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Highway Salting Influences on Ponderosa Pine Seedlings.

FINAL REPORT

To

Rocky Mountain Forest and Range Experiment Station

On

Highway Salting Influences on Ponderosa Pine Seedlings

By

Donald Bedunah and M. J. Trlica

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INTRODUCTION

Winter recreational demands for skiing, snowmobiling, cross-country ski touring and winter camping have significantly increased useage of western forests during the past several years. Ever-increasing winter recreational demands for use of forested lands in the western United States results in more vehicle-use days within these forests. With increased demand for winter recreation, highway maintenance in the form of snow removal, sanding and salting will probably be used more extensively in the future to provide access to resort developments, second homes, and remote forested areas. Where salting of roadways is utilized, significant amounts of salts enter the surface waters on or near these roadways. These salts decrease water quality and can directly or indirectly injure vegetation near the roadway. Salty water can be splashed on foliage by snow removal equipment or vehicles causing direct foliar injury, or the salty water can enter the soil causing decreased osmotic potential of the soil solution. Reduced water uptake by plants can be caused by decreased osmotic potential of the soil water solution. Either direct or indirect ionic effects of salts can reduce productivity of vegetation near the roadway. In addition, visual injury of vegetation by salts is aesthetically unpleasing.

Most studies on road salting in the United States have dealt with determining if salts were the causative agent of tree decline along highways. These investigations have dealt mainly with the occurrence of visual symptoms such as leaf necrosis and tip burn, and with levels of foliar chloride and sodium. The rate of photosynthesis

may be a more important indicator for determining the injury that salts have on vegetation than is the amount of foliar injury or the concentration of specific ions in the foliage.

This study was, therefore, undertaken with the primary objective of determining if different concentrations of sodium chloride administered as a foliar spray and as irrigation treatments caused changes in the carbon dioxide (CO_2) exchange rates of Ponderosa pine (Pinus ponderosa Laws) seedlings. Ponderosa pine was used as the experimental organism because of its importance in the western forested lands of the United States. A decrease in net photosynthesis without outward signs of injury could be occurring as a result of salt concentrations. This decrease in photosynthesis might lower plant vigor, leading to injury from diseases, insects or other causes.

A secondary objective of this study was to compare the effects of isoosmotic solutions of polyethylene glycol (PEG) and sodium chloride on ponderosa pine seedlings. This comparison was made to determine if salt injury was caused by osmotic potentials or a specific ion effect of sodium or chloride, or a combination of sodium and chloride.

REVIEW OF LITERATURE

Bernstein and Hayward (1958) defined salinity as the presence of excessive concentrations of soluble salts. They considered the lower limit for a saline condition as that level which caused a measureable or significant decrease in plant growth, yield, or quality of the crop. Salinization of soil creates extremely unfavorable conditions for plant growth. Salt accumulation increases the osmotic pressure of the soil solution which tends to restrict the uptake of water by plants. An element present in high concentrations of a saline soil may exceed the concentration of some essential nutrients by 10- or 100-fold in the case of a macro-nutrient, and by much larger ratios in the case of micro-nutrient elements. With such large variations in ionic ratios, effects of salinity on nutritional balances would not be unexpected (Bernstein and Hayward, 1958). Salts may also act as specific poisons. The specific ions of salts may have physiological effects that do not appear as visible symptoms.

Salt Tolerance Studies

Plants vary widely in their nutrient requirements and capacity for uptake of different ions. Therefore, they have different responses and sensitivities to different saline conditions. Monk and Wiebe (1961) determined salt tolerances of 28 species of woody and herbaceous plants. Salt hardiness, the ability of cells or protoplasm to survive elevated internal salt concentration, was measured in this study by plasmolysis and tetrazolium tests.

Ponderosa pine was placed into an intermediate category of salt tolerance.

Carleton (1962) studied salt tolerance responses of four species of pine, Pinus thunbergii, P. radiata, P. resinosa, and P. taeda. At any given salt concentration, leaves of salt-sensitive species had higher concentrations of salt, particularly sodium and chloride, than did those that were more salt tolerant. The concentration of ions in needles (leaves), particularly sodium and chloride, rose sharply as the salinity concentration in the root medium was increased. Westing (1969) found that sodium-sensitive plants are harmed when sodium reaches about 0.3 to 0.5 percent of the dry weight of leaves.

In a greenhouse study, Werkhoven et al. (1966) utilized salinity levels of electrical conductivity (EC_e) equal to 1, 4, 7, and 10 mmhos/cm at 25°C to test salinity tolerance of seedlings of Colorado spruce (Picea pungens), Scots pine (Pinus sylvertris), caragana (Caragana arborescens), and Siberian elm (Ulmus pumila) at two levels of soil moisture. Emergence, survival, plant height, and dry-matter yield were used as the response criteria. The deciduous species were more salt tolerant than were the coniferous species. In terms of survival, an EC_e value of between 7 and 10 mmhos/cm appeared to be critical for caragana and elm in a soil with a moisture content midway between the wilting point and field capacity. The corresponding values for spruce and pine were closer to 4 and 6 mmhos/cm, respectively. Seedling survival was markedly improved by maintaining the soil moisture level at field capacity.

Absorption of Salts

Kramer (1969) described the absorption of salt by plants as involving several steps. The principal ones being the movement of ions from the soil solution to root surfaces, then through root cells into the xylem, and finally translocation of the ions from the roots to the shoots. Salts are carried from one point to another by the movement of soil water. Frost formation impedes water movement (Prior and Berthouex, 1967). However, Westing (1969) found that salts are capable of percolation into the soil horizon even when the ground is frozen.

Salt uptake by roots is in part passive, but largely it is an active (i.e., metabolic) and a partially selective process (Westing, 1969). Experiments with radioactive tracers indicate that ions enter roots and move to the shoot most rapidly in the same region through which most water enters (Kramer, 1959). Kramer (1959) suggested that ions are accumulated in the cytoplasm and move from cell to cell through the plasmodesmata which connects the cytoplasm of adjacent cells to form a continuous system, the symplast. Inward movement of salt from the epidermis to the xylem might occur by active transport, diffusion and mass movement in the transpiration stream. A type of active transport mechanism occurs, resulting in the accumulation of salt in the xylem sap (Kramer, 1959). Kramer (1959) found no barrier to inward movement by diffusion, at least as far as the endodermis. If ions move by diffusion, they could also be carried by mass flow in the transpiration stream.

Active transport undoubtedly plays an important role in movement of salt into the roots. However, considerable salt uptake probably occurs by diffusion and mass flow, especially in older roots and rapidly-transpiring plants (Kramer, 1959). Regardless of whether salts move actively or passively, their movement will be affected by the condition of the cytoplasm through which the salts must pass. Temperature, aeration, and respiratory inhibitors affect passive water movement by modifying the resistance of roots. These factors will also affect the passive movement of ions carried in the water, in addition to affecting the active transport mechanism (Kramer, 1959).

Sodium and chloride are also absorbed through plant leaves. Wittwer and Teubner (1959) found that sodium was among the most readily abosorbed cation by plant leaves and was probably moved directly through the cuticle. Boyce (1954) studied salt spray communities near the ocean and found an increasing chloride gradient occurred in individual plants from the leeward to the windward side. Some chloride was translocated to the leeward twigs, but not in concentration sufficient to cause injury. Boyce (1954) believed that the chloride ion, after foliar absorption, was rapidly translocated to the apices of the leaves and twigs. Accumulation in these regions was of an injurious concentration.

Influence of Excess Salts on the Soil Environment

The soluble salts that effectively contribute to soil salinity consist mostly of cations of calcium, magnesium, and sodium, as well as anions of chloride and sulfate. Excess exchangeable sodium

affects both the physical and chemical properties of soils, and may greatly alter the suitability of the soil for plant growth. A soil is termed a sodic soil when it contains enough exchangeable sodium to interfere with growth of most crop plants (Richards, 1954). For purposes of definition, sodic soils are said to have a sodium saturation equivalent of 15 percent or more of the cation exchange capacity (Richards, 1954). Soils are classified as saline when the specific conductivity of a saturation extract is greater than 4 mmhos/cm at 25°C. A saline-sodic soil has a sodium saturation of greater than 15 percent and a specific conductivity of greater than 4 mmhos/cm at 25°C (Richards, 1954).

The marked influence of exchangeable sodium on the physical properties of soils is associated primarily with the behavior of clay and organic matter, where most of the cation exchange capacity is concentrated (Black, 1968). Soil particles have a greater tendency to separate in the presence of exchangeable sodium rather than calcium, because of the difference in forces of attraction and repulsion associated with the two ions. Interparticle bonding, through distribution of charges of the cation between adjacent particles, is less with monovalent sodium than with divalent calcium. A repulsion of particles caused by mutual repulsion of exchangeable cations is greater with sodium than with calcium because of the larger diameter of the hydrated ion of sodium around soil particles (Black, 1968). In the presence of excess salt, even sodium-saturated clays are deflocculated. These deflocculated soils become poorly aerated and water flow is greatly decreased.

Nutrient deficiencies of plants is often caused by a saturation of ions from the salts on the soil colloids. Excess sodium may induce deficiencies of other cations, particularly calcium and magnesium. The action appears to be two fold. Since sodium is comparatively loosely held in an exchangeable form, the ions are easily released to the soil solution. Secondly, at high pH values usually associated with excess exchangeable sodium, the soil solution contains bicarbonate and carbonate ions that tend to precipitate calcium and magnesium as carbonates.

Solov'ev (from Strogonov, 1964) observed a potassium deficiency in pumpkin (Cucurbita pepo) and sweet clover (Melilotus alba) plants subjected to sodium chloride (NaCl) salinization. Further experiments led him to conclude that the main cause of NaCl-induced growth inhibition was the difficulty in uptake of mineral nutrients caused by competition with sodium. This conclusion was favored by the restoration of potassium supply and normal plant growth by placing an isolated root strand in a nutrient solution. When the root strand was placed in pure water, growth was not restored. Zhukovskaya (from Strogonov, 1972) found that both chlorides and sulfates caused a decrease in content of total and inorganic phosphorus in tomatoes (Lycopersicon esculentum).

Road Salting

Most studies on the effects of road salting in the United States have dealt with determining if salts were the causative agent of tree decline along highways. Davidson (1971) believed that sufficient salts persisted near roadways that received salt to affect the vegetation at least by causing osmotic stress and by altering nutrient balances.

Salt damage to roadside vegetation was studied in the western United States in the winter of 1972 and 1973, when California foresters observed that trees along highways in the Lake Tahoe Basin were declining and dying (Scharpf and Srago, 1974). Trees along the roadsides had sodium and chloride concentrations several times greater than trees not exposed to road salting. The concentrations of these ions in foliage tissue were directly related to damage symptoms.

Holmes (1961), during a seven-year test on applications of sodium chloride and calcium chloride to sites of red maple (Acer rubrum), black oak (Quercus velutina), white oak (Quercus alba), pignut hickory (Carya glabra), black birch (Betula nigra), ash (Fraxinus sp.), and white pine (Pinus strobus), found that road salting probably did no great harm to trees in Massachusetts. Sugar maples (Acer saccharum), which received run-off water from road surfaces, contained three to six times as much chloride as did leaves from control plants. However, trees treated only in the winter showed no symptoms of injury. It was believed that chlorides from the soil were accumulated in the leaves and were removed from the trees when the leaves fell each autumn.

In a later study, Holmes and Baker (1966) determined that sugar maples with little or no foliage injury had low foliar chloride levels (0.05-0.60 percent dry weight), whereas those with severe leaf injury had high foliar chloride levels (about one percent). The correlation between foliar chloride level and foliar injury supported evidence that salts injured and even killed sugar maple trees. However, many roadside maples showed low foliar chloride levels and little foliar

injury, supporting evidence that road salting did little harm to these maples.

Kotheimer (1967) conducted extensive field surveys to assess salt injury and assign damage-rating values to sugar maple, Norway maple (Acer platanoides), and red pine (Pinus resinosa) growing within close proximity to highways in New Hampshire. A brief survey and analysis of foliar tissues of white pine and red pine implicated that the use of highway salt was probably responsible for the decline of roadside pines. Sugar maple decline was defined as a syndrome involving foliar damage through the excessive accumulation of chlorides and simultaneous nutrient imbalance promoted by sodium. Maple decline was characterized further by the gradual depression of the growth processes and the accumulation of nitrogeneous metabolites.

Walton (1969) determined that both calcium chloride and sodium chloride were phytotoxic when applied to soil around young Norway maple trees. When applied in the spring, the chloride ion of these salts injured the foliage directly. The injury that subsequently developed when salts were applied in the winter was the result of a combination of the direct toxicity of the chloride ion along with other factors that caused water stress in the plant. Calcium chloride treatments increased the foliar calcium and chloride contents, decreased foliar potassium content, and caused essentially no change in sodium content. The sodium treatment increased potassium, sodium and chloride content. Calcium chloride caused extensive foliar necrosis and was the most toxic of the two salts.

Hutchinson and Olson (1967) found that levels of sodium and chloride ions in soils along highways in Maine increased considerably where road salting had been used. The sodium and chloride levels were greatest near the edge of the road embankment and decreased with distance from the highway. Levels of both ions increased with the length of time for which salting had been practiced. Since high concentrations of sodium ions have an adverse effect on soil physical properties, such as dispersion of colloidal particles, it appeared that the sodium levels found at some sites in this study would suggest that salt applications were resulting in poorer drainage of the soils along the highways. Furthermore, the sodium and chloride levels present in these soils were rapidly approaching a point where they might be toxic to some of the desirable vegetation species growing in the area.

Serious salt damage to vegetation was observed in the Washington D.C. area during the summer of 1967 (Wester and Cohen, 1968). The damage was most prevalent for Kentucky bluegrass (Poa pratensis), California privet (Ligustrum ovalifolium), American elm (Ulmus americana), sugar maple, Quebec linden (Tilia neglecta), and Canadian hemlock (Tsuga canadensis). Snowfall during the preceeding winter was twice that of normal and required additional salt for its removal. This increased salting, coupled with a dry spring, allowed little opportunity for the salt to be leached out of the soil. The accumulation of salt in the soil was believed to have caused plasmolyzed feeder root tissue and prevented adequate water absorption. This resulted in injury to foliage and retarded shoot development.

Button and Peaslee (1967) found that the concentrations of sodium and chloride in trees increased with increasing exposure to pavement drainage. Under continued exposure to low levels of salt, there could be a gradual accumulation of toxic ions, depression of uptake of nutrient ions, and subsequent slow degeneration of large trees. Chloride increased to near toxic levels in trees without causing immediate leaf burn symptoms. Conversely, during the final stages of decline, trees had lower concentrations of chloride in the leaves than did apparently healthy trees.

Smith (1970) sampled twig and needle growth of white pine to determine sodium and calcium contamination along the Connecticut Turnpike. Many samples had sodium concentrations greater than one percent on a dry weight basis, including several trees located more than 20 m from the road. A few samples had sodium contents equal to or greater than 1.9 percent. Calcium contents did not exceed normal calcium levels for white pine. It was suggested that white pine should not be planted closer than 35 m from high speed roads in order to avoid injury from airborne salts.

Smith (1970) stressed the fact that in salt contamination studies of roadside trees, salt spray had not been studied as a major factor in ion uptake although aerial salt transport may occur over considerable distances on either side of the highway. A disparity between sodium levels on the north and south side of a highway was most probably caused by the pattern of prevailing winds.

Considerable salt is introduced into the air adjacent to high speed highways in the form of water particles from a wet highway and

dust particles from a dry roadway by the churning action of tires and air currents generated by moving vehicles (Hofstra and Hall, 1971). Hofstra and Hall (1971) noted greater foliage damage to white cedar (Thuja occidentalis) and several species of pine on the downwind side of highways in southern Ontario. Foliar injury occurred up to 120 m from the highway on the south side of the highway because of predominant northwesterly winds during the winter.

Hall et al. (1972) used both lab experiments and roadside analyses to determine the effect of saline foliar spray on white pine. There was no foliar browning as long as the temperature was kept below 1.5°C, but foliar browning became obvious two days after trees had been placed in a greenhouse at 15°C. The amount of injury and the sodium and chloride content of the leaves increased as the number of spray applications increased. The ratio of sodium to chloride was very close to unity, suggesting that these ions moved into the foliage with equal facility. There was no apparent foliar injury near roadways until milder temperatures of spring were reached. Then damage quickly became visible. The amount of necrotic foliage on trees declined over the ensuing months as a result of premature abcission of leaves injured by salt spray.

Sucoff et al. (1975) demonstrated that NaCl spray was the primary cause of foliar browning of Pinus resinosa along Minnesota roads receiving winter salting. Damaged needles were found to have high chloride and high sodium levels. Symptoms produced by spraying NaCl on forest trees appeared identical with highway symptoms. They found greater injury caused by foliar spray than from salt applied near the base of trees.

Zelazny and Blaser (1970) took foliage samples from mature trees along a roadside representing a range of increasing injury symptoms and chloride content. The levels of accumulated ammonia and of various amino acids were directly proportional to the injury symptoms and chloride content. Whether the compounds formed were end products of pathways operating to remove the injurious ammonia or simply represented an accumulation of normal metabolites was not determined.

Physiological Effects

Previous road salting experiments have dealt primarily with the occurrence of outward symptoms such as leaf necrosis and tip burn. However, the photosynthetic rate may be an important indicator for assessing injury that salting is causing to vegetation.

Black (1968) listed three theories to account for different aspects of the detrimental effects of soil salinity: the water-availability theory, the osmotic-inhibition theory, and the specific-toxicity theory.

Water-availability theory. Soluble salts in saline soils decrease the water solute potentials of the soil water. The availability of water for plant uptake is thereby decreased. Therefore, plants may suffer a deficiency of water.

Eaton (1941) tested this theory by dividing the roots of corn plants (Zea mays) equally between two nutrient solutions having different solute potentials. The solute potential of the more dilute solution was -0.3 bars and that of the more concentrated solution was -1.8 bars. Uptake of water by the plants amounted to 319 and 171 ml

for the two solutions, respectively. This simple experiment demonstrated a marked reduction of water uptake at lower solute potential.

Hayward and Spurr (1944) found that the rate of water uptake by corn plants decreased with increasing concentration of solutes. This decrease in water uptake was independent of the nature of the solutes where all concentrations were expressed in terms of the solute potential of the solution.

Black (1968) questioned the validity of the water-availability theory as an explanation for the nonspecific detrimental effects of soil salinity on plant growth on the basis of two kinds of experimental observations. First, if salinity inhibited uptake of water by plants the plants should lose turgor and transpiration per unit of leaf area should be reduced. Bernstein (1961) referred to unpublished data by W. L. Ehrler showing that transpiration per unit of leaf area was not affected by salinity. He also referred to Eaton's (1942) data which showed that although salinity decreased transpiration, the major part of the decrease could be accounted for by the associated decrease in weight of the plants.

Secondly, Black (1968) questioned the validity of the water-availability theory on the basis that experimental evidence did not support the postulate that the solute potential contributed by the salts reduced the difference between total suction of water in plants and soil.

Slayter (1961) grew plants in a nutrient solution with a solute potential of -0.7 bars and then transferred a plant to similar solutions with enough sodium chloride to give an additional -5 or -10 bars

of solute potential. Initially the plants lost water and wilted in accordance with the water-availability theory. However, the plants recovered from wilting within 28 hours (h) and essentially regained the original content of water. Accompanying the recovery from wilting was an uptake of solutes and a decrease in solute potential in the plant sap that was approximately equal to the -5 and -10 bars of solute potential added to the external solution by sodium chloride. Adjustment made by the plants, principally in uptake of solutes, had eliminated the decrease in water availability to the plants.

In a general sense, the water-availability theory postulates that the action of salts in a saline soil is on the soil water and that this action has an indirect effect on water supply inside the plant. The foregoing evidence indicates that the water-availability theory does not apply, at least in the form originally conceived.

Osmotic-inhibition theory. According to the osmotic-inhibition theory, plant growth is inhibited by the excess solutes taken up from a saline media. The osmotic-inhibition theory thus postulates that the salts act inside the plant, but it does not specify what causes the inhibition of growth. The inhibition could result in part from water deficiency in a sense different from that envisioned in the water-availability theory.

Levitt (1972) stated that if salt lowers the external cell water potential below that within the cell, it exposes the cell to a secondary water deficit stress. To distinguish this stress from drought stress, and because it leads to an osmotic dehydration strain, it is called an osmotic stress. This secondary water deficit stress has

also been called physiological drought. Because of this osmotic effect, halophytes show a quantitative relationship between the osmotic value of their sap and that of the root medium. This is usually caused by the absorption of large quantities of salt from the external medium, thus causing a higher total osmotic concentration inside the cell than outside. Nevertheless, the salt concentration inside the cell will usually be slightly lower than that outside the cell because of the presence of organic solutes within the cell (Marre ans Servettaz, 1959). The osmotic values of the leaves of salt bush (Atriplex canescens), for instance, varied directly with that of the solution in which the plants were growing. A constant absolute value of about -12 bars greater than that of the range of treatment solutions was observed from the leaves (Ashby and Beadle, 1957). As a result leaf osmotic pressures ranged from 30 times that of the nutrient solution for control plants to only twice that of controls for plants growing in solution having 600 mEq/liter. Beadle et al. (1957) found that this relationship was also true in natural ecosystems. In soils with chloride contents varying from 19 to 9250 parts per million (ppm), the leaves of the Atriplex plants had chloride contents ranging only from 9.6 to 13.6 percent of their dry weights, respectively. In the case of Moqochrysis lutheri, the osmotic balance was mainly achieved by changes in cell content of cyclohexanetetrol (Craigie, 1969). Even in the case of extremely salt-sensitive plants, such as strawberry (Fragaria ananassa), the osmotic effect of the salt was the principal factor limiting plant growth (Ehlig and Bernstein, 1958).

Specific-toxicity theory. According to the specific-toxicity theory, soil salinity exerts a detrimental effect on plants through the toxicity of one or more specific ions present in excess. In the osmotic-inhibition theory, plants are able to protect themselves against osmotic dehydration by accumulating the salt (and sometimes a soluble metabolic product) until the total osmotic concentration is in excess of that in the root medium. In the case of specific-toxicity, the more salt that is accumulated the greater is the injury.

Raspberries (Rubus occidentalis), for instance accumulate chloride ions more rapidly than boysenberries (Rubus hispidus) or blackberries (Rubus allegheniensis) and were killed or severely injured (Ehlig, 1964). The chloride from sodium chloride solutions was accumulated by the plants more rapidly than was sodium. Therefore, chloride injury occurred earlier and was more severe than sodium injury. Tagawa and Ishizaha (1963) also concluded that the primary cause of injury to rice (Oryza sativa) by sodium chloride was chloride accumulation in the shoots. Strogonov (1964) found the same thing for cotton (Gossypium hirsutum). The injury depended not on the total salt present, but on the chloride/sulphate ratio. Sodium chloride was increased to an amount approaching the critical level for growth of rice, wheat (Triticum aestiuum) and barley (Hordeum vulgare) grown in sand cultures. Even the largest additions of sodium chloride lowered the leaf water content only by 1.5 percent (Oga and Nishikawa, 1959). The effect was, therefore, not caused by osmotic dehydration.

Staley et al. (1968) found a strong relationship between ponderosa pine tip burn and soil chloride levels in saline soil near Denver,

Colorado. Many other researchers have found a relationship between the amount of chloride in foliar samples and foliar injury where road salting is a common deicing method.

Heiman (1966) believed that sodium rather than chloride was causing the toxic reaction. He based this conclusion on evidence that sodium was retained in roots and caused subterranean damage. It has long been known (Scarth and Loyd, 1930) that maintenance of the normal differential permeability of the cell depends on a balance (about 10:1) between monovalent (K^+ and Na^+) and divalent (mainly Ca^{2+}) cations. When this balance is disturbed by high concentrations of monovalent cations, the permeability of cell membranes increase, leading to injury.

The most common method for distinguishing between secondary osmotic and primary salt injury is to compare the effects of isotonic solutions of the salt with those of organic substances. Carbowax (polyethylene glycol or PEG), for instance, permitted larger yields of bean plants (Phaesolus vulgaris) than those grown in isoosmotic solutions containing ions (Na^+, Ca^{++}, Mg^{++} chlorides)(Lagerwerff, Ogata and Eagle, 1961). At equal osmotic concentrations, sodium chloride depressed the germination of alfalfa (Medicago sativa) seeds much more than did mannitol (Strogonov, 1964). Excised roots showed a similar effect, five times as much growth in mannitol as in sodium chloride solutions of the same osmotic concentration. The toxic effect was apparently caused by the anion rather than the cation. At isoosmotic concentrations of -4.5 bars, Strogonov (1964) found that sodium chloride and especially sodium sulphate significantly

lowered growth and development compared to dextran. This indicated a specific inhibitory effect of the salt over and above any osmotic effect. By use of this method, they found some plants were relatively insensitive to the osmotic effect and were primarily affected by the ions of the salt. Sodium chloride injury to maize (Zea mays), for instance, was primarily caused by the penetration of excessive amounts of the salt into the cells (Lapina, 1966).

Further evidence of a specific injury can be obtained by comparing the effects of different salts. Sodium chloride, calcium chloride, and sodium sulphate depressed the growth of red kidney beans (Phaseolus vulgaris) to the same degree as concentrations yielding the same osmotic potentials (-1.5 to -4.5 bars) relative to the control solution (-0.5 bars)(Gauch and Wadleigh, 1944). Magnesium chloride and magnesium sulphate produced a more pronounced retardation and definitely injured the plants. Similarly, very young sprouts of Sophora japonica required twice as much sodium sulphate or sodium carbonate (0.1 N) as sodium chloride (0.05 N) to inhibit them, in spite of the lower osmotic potential of the sodium chloride solution (Alekperow and Khrzhanovskaya, from Strogonov, 1964). Ende et al. (1975) found that adding NaCl to increase the osmotic pressure reduced yields in all crops studied to a greater extent than adding potassium nitrate, apparently because of specific ion effects. Spotts (1969) produced tip burn in pines treated with a solution of sodium chloride, a calcium-magnesium chloride mixture, and a calcium-magnesium-sodium chloride mixture. Sodium sulphate and sodium carbonate solutions

did not produce typical tip burn symptoms. He found a strong correlation between needle injury and calcium, magnesium and chloride concentration in the tissue. Such differences could only be explained by a greater primary injurious effect of the magnesium ion than the sodium ion, and of the chloride ion than the sulphate or carbonate group.

Levitt (1972) used primary salt stress instead of specific toxicity, and secondary stress instead of osmotic inhibition, to account for the different types of salt injury. He further divided these salt stresses as diagrammed in Figure 1. Primary salt injuries are usually identified by their speed of action. However, the existence of a primary direct salt injury is still in question (Levitt, 1972). Repp (1961) showed that even most halophytes were sensitive to direct salt effects produced by a sudden salt shock. In habitats with marked fluctuations in salt concentration in the rooting medium, only the species with high shock resistance could survive. Furthermore, the young plants that were still low in salt content were more shock sensitive than the older plants (Repp, 1961).

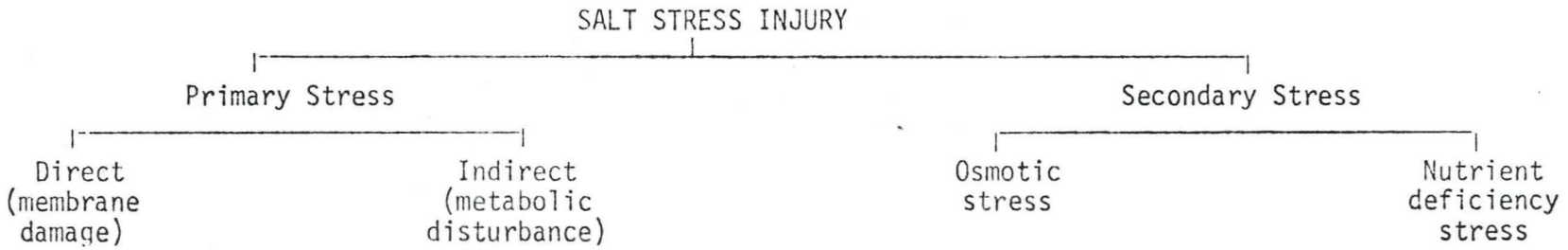


Figure 1. The different kinds of salt stress experienced by plants (Levitt, 1972).

Salt Effects on Metabolic Processes

To understand the nature of the growth inhibition, it is necessary to know what metabolic changes take place in the plant. There have been many reports of salt-induced changes in several metabolic processes such as respiration, photosynthesis, protein synthesis, and nucleic acid synthesis. Enzyme activity has also been reported to be affected by salts as has carbohydrate and nitrogen metabolism. It is therefore usually assumed that salt injury is not caused by a direct effect of the salts themselves, but rather by the indirect effects of a disturbance of one or more of these metabolic processes.

Respiration. Kadota (1962) found that respiration of Pinus thunbergii leaves varied not only with the concentration of a sodium chloride solution, but also with the light condition. The respiration rate of the base parts of the leaves decreased steadily in proportion to increasing concentrations of sodium chloride in both light and dark conditions. The respiration rates of the upper part of the leaves in the light was different from that in the dark and was somewhat stimulated by sodium chloride. This increased rate of respiration of leaves treated with a sodium chloride solution was probably caused by increased sugar content. The increase in sugar content of the leaves resulted from less translocation of sugars to the shoots. Sodium chloride solutions were also found to decrease root respiration as well as reducing the contents of some macro-nutrient elements such as phosphorus, potassium and calcium.

Nieman (1962), working with 12 crop species, found that sodium chloride stimulated respiration per unit leaf area in all species but

pea (Pisum sativum). The stimulation was usually greater and occurred at lower levels of sodium chloride concentration for sensitive species. In the radish (Raphanus sativus), the increase in respiration roughly paralleled the reduction in growth.

Several researchers have found an increase in respiration caused by the addition of a salt (Siew and Klein, 1968; Livne and Levin, 1967), but other researchers have found that salinity decreased respiration (Strogonov, 1964; Boyer, 1965). Inorganic salts can stimulate oxygen consumption of plant tissue, and more specifically it was Dihydronicotinamide adenine dinucleotide phosphate oxidase activity which was stimulated dramatically by salt additives (Hackett, 1961). Miller and Evans (1956) found stimulation of cytochrome oxidase by addition of small amounts of salts.

Photosynthesis. Wallace and Romney (1972) grew big sagebrush (Artemisia tridentata), fourwing saltbush (Atriplex canescens), and shadscale (Atriplex confertifolia) in full nutrient solutions plus 0, 50, 100, 200 or 400 me/liter sodium chloride. Their data indicated that photosynthesis and transpiration decreased for each species in the 200 and 400 me/liter sodium chloride treatments. Big sagebrush also had decreased photosynthesis in the 50 me/liter sodium chloride treatment.

Gale and Poljakoff-Mayber (1970) found that Atriplex halimus exhibited only a slight depression in photosynthetic activity when plants were grown in media of increasing sodium chloride concentrations up to 440 mM (-20 atm). Calculations of mesophyll diffusive resistance showed an initial decrease as plants were cultured in salinized media of approximately 184 mM (-8 atm). When plants were grown at

higher salinities, the mesophyll resistance began to increase. Only at the greater salinities when both stomatal diffusive resistance and mesophyll resistance were increased was there a noticeable decrease in the photosynthetic rate.

Anderson (1962), working with greasewood (Sarcobatus vermiculatus), found that sodium chloride concentration in excess of 450 mM (-21 atm) greatly depressed photosynthesis. El-Sharkawi and Michel (1975) grew alkali sacaton (Sporobolus airoides) and blue grama (Bouteloua gracilis) in salt solutions of known osmotic potentials. Both respiration and photosynthesis decreased with decreasing soil water osmotic potentials. Gauch and Eaton (1942) concluded that in the case of barley, salinity affected the utilization of photosynthate rather than photosynthesis itself.

Tiku (1976), studying two halophytic species, found that application of NaCl increased the biomass and succulence of Salicornia rubra and decreased that of Distichlis stricta. The carbon dioxide (CO_2) uptake of both species increased with increasing light intensity and was strongly influenced by the application of NaCl. All concentrations of NaCl (0, -8, -16, and -32 bar osmotic potential) stimulated the CO_2 uptake of Salicornia, but decreased that of Distichlis. The rate of CO_2 uptake of both species declined with time at all levels of salt treatment. Salicornia had a more rapid decline in CO_2 uptake in the absence of NaCl, whereas that of Distichlis was relatively unaffected. Tissue sodium concentration of the two species increased with increased NaCl concentration in the medium, but the increase in Salicornia was 3.0 to 5.5 times greater than that of Distichlis. The tissue osmotic

potential of both species decreased with decreasing osmotic potential of the medium. In Salicornia, the tissue osmotic potential decrease was concurrent with an increase in CO_2 uptake; whereas the CO_2 uptake of Distichlis declined with decreasing osmotic potential of the medium and a decrease in tissue osmotic potential. It was concluded by Tiku that Salicornia required NaCl for its normal development; whereas Distichlis was adversely affected by it. Both species were adapted to osmotic stresses caused by natural osmotic agents like NaCl, but were not very well adapted to artifical osmotic agents such as ethylene glycol.

There is evidence that salts can cause considerable change in leaf pigments. Some scientists believe that salinity destroys chloroplasts and alters their size and numbers. Strogonov (1964) found that chlorophyll formation was depressed at high chloride concentrations. More recent evidence showed that chlorides reduced the number and size of chloroplasts, causing chlorosis in potato leaves (Solanum tuberosum) (Galaktionov, 1963 from Strogonov, 1972). Other researchers maintain that the chloroplasts are not destroyed under salination but are degraded (Koverga, 1956 from Strogonov, 1972). Changes in pigment metabolism could be the cause of changes in the rates of photosynthesis that many authors have found.

MATERIALS AND METHODS

Salt Toxicity Test 1

Ninety ponderosa pine seedlings were planted in 3.75-liter cans in July, 1975. The seedlings had been grown in tar paper containers for two years at the Colorado State Forest Service Nursery in Fort Collins, Colorado. Seeds for these trees had been collected from the Pike National Forest, Colorado. The soil used in planting the seedlings was a 1:1 mix (volume:volume) of forest humus and mountain mineral soil obtained from a ponderosa pine site in the Roosevelt National Forest, Colorado.

Pine seedlings were placed in a lath house on the Colorado State University campus and were allowed to become well established in their containers prior to treatment. To simulate the effects of road salting on the roadside environment, seedlings were irrigated and the foliage sprayed with differing concentrations of sodium chloride solutions (Table 1). A complete factorial design, with CO_2 exchange measurements randomized within blocks, was used with five replications (pots) for each treatment.

On December 31, 1975, the first salt irrigation and foliar spray treatments were initiated. Salt spray and irrigation treatments were conducted on a five-day schedule through the winter. Foliar spray was accomplished by using a hand-held spray bottle. Each pine seedling receiving a foliar spray treatment was sprayed ten times (approximately 7 ml) on each date. Irrigations consisted of watering with 200 ml of a sodium chloride solution, which allowed for a small amount of percolation.

Table 1. Treatment levels of sodium chloride solutions (ppm) utilized in Salt Toxicity Test 1.

Foliar spray - Irrigation[1]
0 - 0
3000 - 0
6000 - 0
9000 - 0
0 - 3000
3000 - 3000
6000 - 3000
9000 - 3000
0 - 6000
3000 - 6000
6000 - 6000
9000 - 6000
0 - 9000
3000 - 9000
6000 - 9000
9000 - 9000

[1]Control plants were irrigated with distilled water and received no foliar spray.

The final treatment day was March 18, 1976. Plants had been treated 16 times by this date. Treatments were discontinued because of severe foliar damage to plants receiving the most concentrated sodium chloride irrigations (9000 ppm). All seedlings were then watered as needed with distilled water to keep the soil near field capacity until the completion of the study.

On May 30, 1976, seedlings were placed in the Range Science greenhouse in order to maintain more stable environmental conditions. Each seedling was placed in a growth chamber under constant light and temperature (20°C) at least 24 h before gas exchange measurements were initiated.

Carbon Dioxide Exchange Measurements

A closed system of gas exchange designed by Ronco (1969) was used to determine photosynthetic and respiration rates of seedlings. Basically, this closed system consisted of three components: (1) an assimilation chamber with very sensitive temperature control that allowed maintenance of constant temperatures from approximately 15 to 40°C, $\pm$ 2°C, (2) a bank of seven 300W reflector spotlights capable of producing irradiances of up to 1.54 langleys per minute between 400 and 700 nm after being filtered through an eight-cm deep continuous flow water bath for removal of much of the infrared radiation, and (3) an infrared gas analyzer (IRGA) which automatically and continuously determined the concentration of carbon dioxide within the chamber.

The closed CO_2 exchange system is diagrammed in Figure 2 and is pictured in operation in Figure 3. A fan provided continuous air circulation within the assimilation chamber. This air circulation reduced

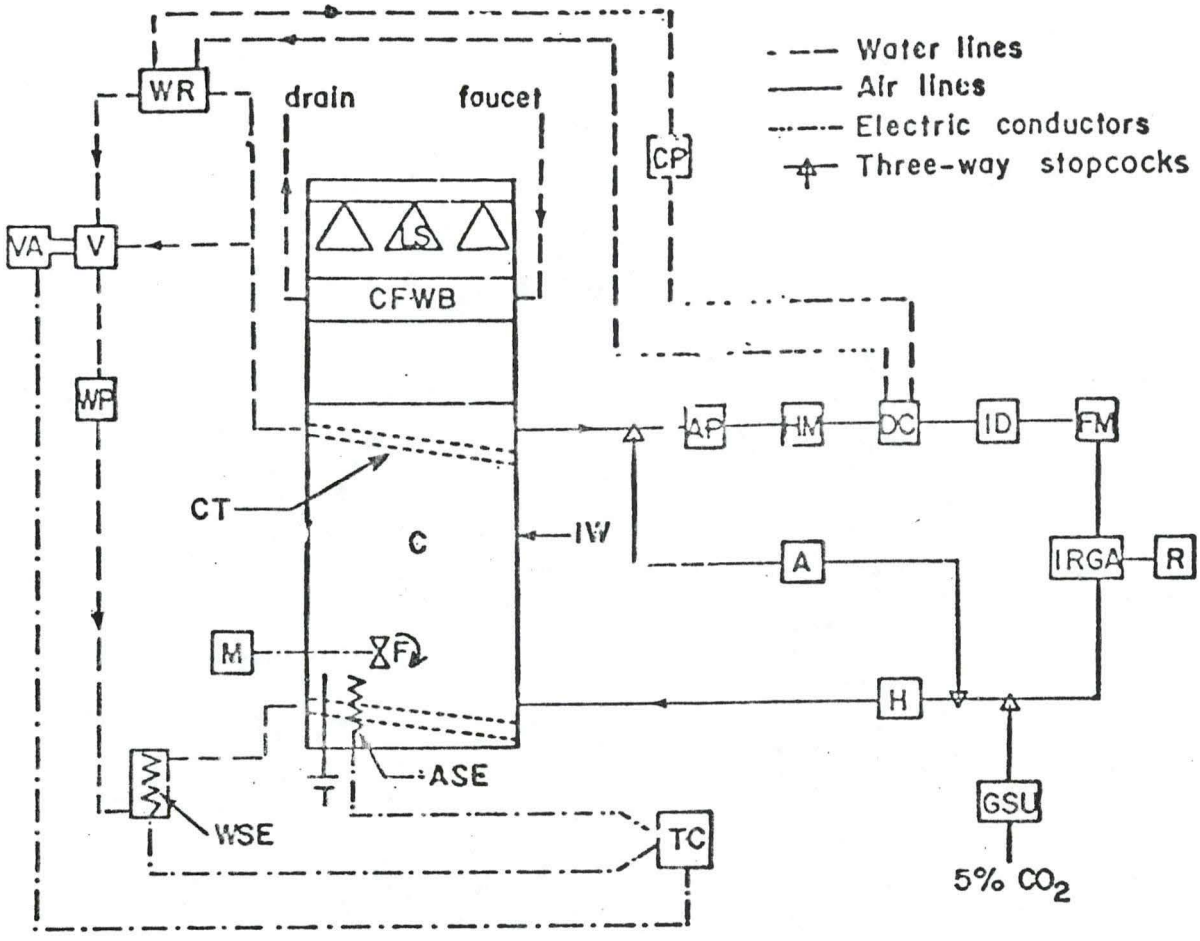


Figure 2. Schematic diagram of CO_2 exchange system developed by Ronco (1969) for measuring photosynthetic and respiration rates for Salt Toxicity Test 1. The various discernible components are the light source (LS), temperature controller (TC), assimilation chamber (C), gas injection unit (GSU), and indicting drierite column (ID).

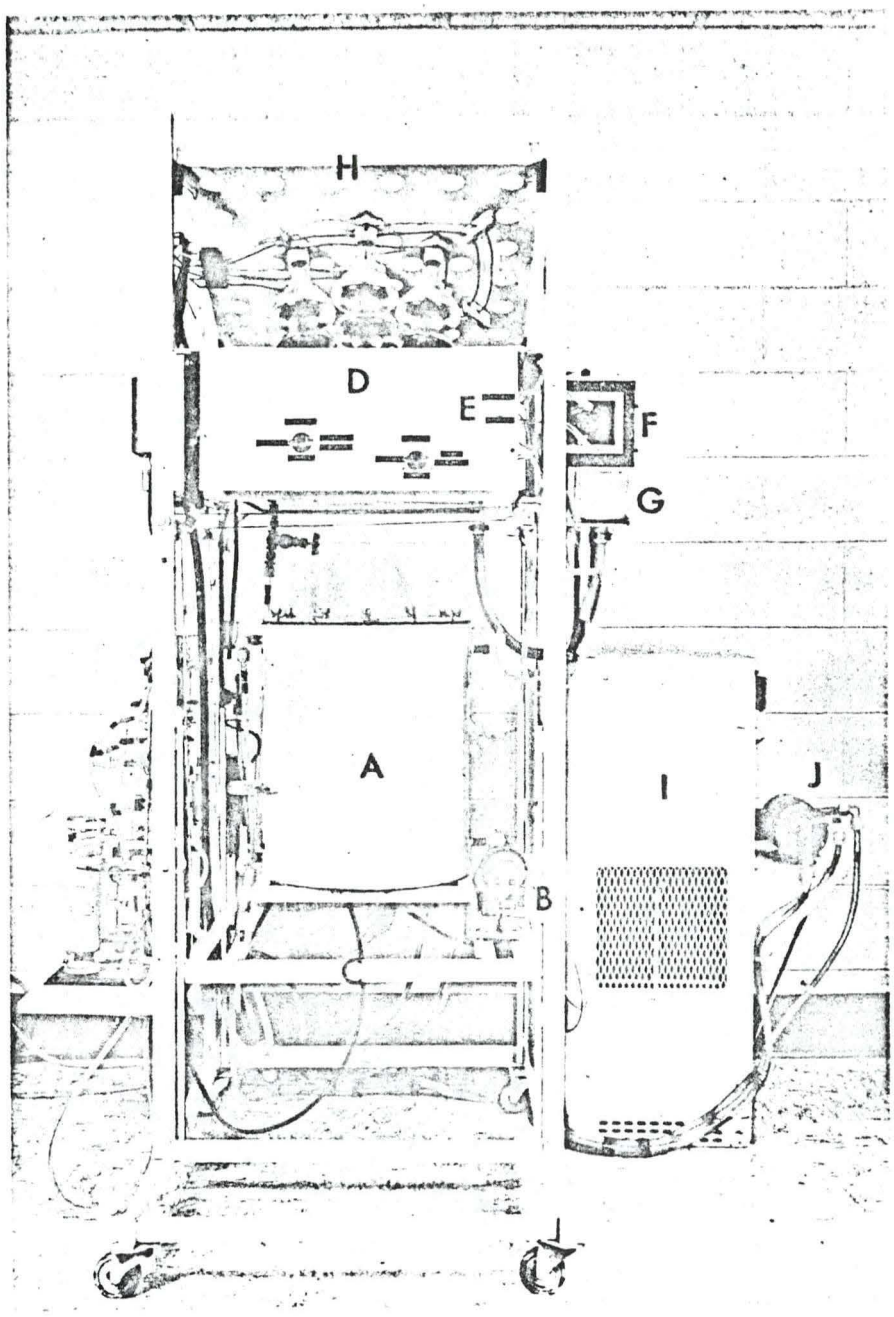


Figure 3. Front view of closed-system apparatus for measuring carbon dioxide exchange of tree seedlings.

A - plant chamber
B - air pump
C - adjustable shelf
D - control panel with 3-way stopcocks of calibration system
E - inlet and outlet tubing to flow-meter and gas analyzer
F - humidity meter
G - water-level control of heat filter
H - light bracket raised to show lamp arrangement
I - refrigeration unit and water reservoir
J - coolant circulating pump

variation in rate determinations by mixing chamber air and removing the possibility of air pockets having different CO_2 concentrations. The temperature control system utilized a modified drinking fountain cooler as a coolant reservoir. A three-way valve regulated the amount of coolant circulated through 12 m of copper tubing between the walls of the assimilation chamber, thus maintaining the desired air temperature within the chamber. The three-way valve was electronically controlled by both an air temperature thermistor in the chamber and a water temperature thermistor in the coolant line. This dual temperature control provided a high degree of temperature sensitivity to the system.

Experimental Procedures

The CO_2 exchange system was a closed system, therefore the CO_2 concentration in the system could not be maintained at one level. Photosynthesis caused a decrease in the CO_2 concentration of the system, and conversely, the CO_2 concentration of the system was enhanced by respiration. Therefore, steady states of photosynthesis and respiration were measured by steady rates of CO_2 concentration decreases or increases, respectively. The rate was recorded as a diagonal straight line on a strip chart recorder.

The determination of photosynthetic rates of plants requires that only the CO_2 exchange of the aboveground portions of the plant be taken into consideration. This provides the net photosynthetic rate (in light) and requires the exclusion of the belowground contribution of CO_2. According to Zelitch (1971), net CO_2 assimilation (sometimes called "apparent" photosynthesis) is equal to the gross photosynthesis (sometimes referred to as "true" photosynthesis) minus the loss from

respiration. More commonly, net photosynthesis (Pn) equals gross photosynthesis (Pg) minus aboveground respiration (AGR). Net photosynthesis was measured as a CO_2 assimilation rate in the light and foliage respiration was measured as CO_2 production in total darkness.

Contribution of CO_2 to the system from the soil was excluded by sealing the soil-atmosphere interface with a mixture of parafin and heavy mineral oil. A heated 3:1 mixture of mineral oil and parafin was poured onto the soil surface of the can and allowed to harden to insure an air-tight seal. Brown (1974) found mineral oil to be impervious to CO_2 diffusion and had no detectable influence on CO_2 assimilation rates of blue grama for up to 15 h after application. Drain holes in the bottom of the can were sealed with Terostat sealant.

Ponderosa pine seedlings were placed in a Sherer growth chamber at 20°C and under continuous light 24 h before the start of gas exchange measurements. Gas exchange measurements were recorded at 20C° and at a level of irradiance of 1.22 ly/min or in total darkness. Soil moisture, light and temperature were held constant for all measurements. Rates of CO_2 concentration changes were recorded between $\pm$ 40 ppm from ambient air to eliminate errors caused by continuously-changing CO_2 concentrations.

The foliage of the pine seedlings were harvested at the conclusion of gas exchange determinations. The foliage was hand separated into green and non-green materials and oven dried at 60°C and weighed. Net photosynthetic rates were calculated on the basis of dry weight of green photosynthetically-active material (DWG) and dry weight of total aboveground biomass of both photosynthetically- and non-photosynthetically-

active leaf material (DWT). Stems were not included in weight determinations.

All photosynthetic and respiratory rates determined in Salt Toxicity Test 1 were computed by the equation:

$$Pn \text{ or } AGR = [(MVT_1P/LT\ P_1)(\Delta ppm/h \cdot 10^{-6})]/DWG \text{ or } DWT,$$

where: Pn = net photosynthesis (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$) on dry weight green (DWG) or total aboveground foliage biomass basis (DWT).

AGR = aboveground dark respiration (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$)

M = mole weight of CO_2 (44,010 mg)

V = volume of closed system (34.48 liters)

T_1 = 273°K

P = average barometric pressure (635 mm Hg)

L = mole volume of CO_2 (22.414 liters)

T = chamber air temperature (°K)

P_1 = standard barometric pressure (760 mm Hg)

$\Delta ppm/h$ = CO_2 exchange rate in parts per million per hour converted to the volume fraction of CO_2 by multiplying by 10^{-6}

DWG = dry weight green aboveground biomass (g)

DWT = dry weight total green and non-green aboveground biomass (g)

Salt Toxicity Test 2

Salt Toxicity Test 2 was designed to delineate between osmotic and ionic effects of sodium chloride on net photosynthesis and dark

respiration of ponderosa pine seedlings. Delineating the osmotic and ionic effects of salts on plants is difficult because plants respond to both effects simultaneously. However, by using isoosmotic solutions it is possible to determine if the major effect is osmotic or ion toxicity. By using polyethylene glycol (PEG) it is possible to create equal osmotic concentrations as sodium chloride solutions and to test for osmotic stress or ion toxicity. Mexal and Reid (1973) showed that PEG 4000 was not toxic and unabsorbed by ponderosa pine. Therefore, this experiment was designed to detect almost immediate effects of sodium chloride or PEG on gas exchange rates.

On January 12, 1976, 30 two-year-old ponderosa pine seedlings (from the same source as seedlings from Salt Toxicity Test 1) were planted in 3.75-liter plastic pots containing a washed river sand. A sand culture was used instead of soil to eliminate interactions between the salinity treatments and the soil. The seedlings received continuous light in the Range Science greenhouse on the CSU campus until they broke dormancy. Temperature in the greenhouse varied from 17 to 32°C. Seedlings were watered with 150 ml of 0.25x Hoagland's nutrient solution approximately every third day. This 150 ml of solution allowed for a small amount of percolation from the pot. Occasionally the sand cultures were watered with 250 ml of distilled water to leach out any ions that may have accumulated in the pots.

The seedlings were allowed to grow in the greenhouse environment for approximately five months before gas exchange measurements were made. Then the seedlings were transported to a growth chamber 24 h before gas exchange measurements were to begin. The growth chambers

were set for continuous light and at a constant temperature of 18°C. The light source in the growth chamber was capable of producing irradiances of up to 0.27 ly/min between 400 and 700 nm..

Carbon Dioxide Exchange System Description

The CO_2 exchange system utilized for photosynthetic and respiration rate determinations was an open system. This system differs from a closed system in that air is continuously flowing into and out of the cuvette from the outside ambient atmosphere. Basically, the open system consisted of two components: (1) an assimilation chamber or cuvette, and (2) an infrared gas analyzer.

The open CO_2 exchange system is diagrammed in Figure 4 and is pictured in operation in Figures 5 and 6. A fan provided continuous internal air circulation within the cuvette. The cylindrical cuvette was built with 317 mm plexiglass by the Engineering Research Center at Colorado State University. The cuvette was 35 cm high and 20 cm in diameter. The fan was made with a Cer-Mag No. 15 D.C. motor and a model airplane propeller. The power to the motor was supplied from a 1.5 V battery.

The cuvette temperature was measured by a thermistor and controlled by the growth chamber (Sherer Gro-Lab Model #CEL257HL). Light was supplied by the growth chamber with an irradiance of 0.27 ly/min. Humidity in the growth chamber was held constant (dew point temperature = 7°C) and no attempt was made to control cuvette humidity.

Carbon dioxide differentials between ambient air and air withdrawn from the cuvette were measured with a differential infrared gas analyzer (IRGA). The CO_2 exchange rates were recorded on a strip chart recorder.

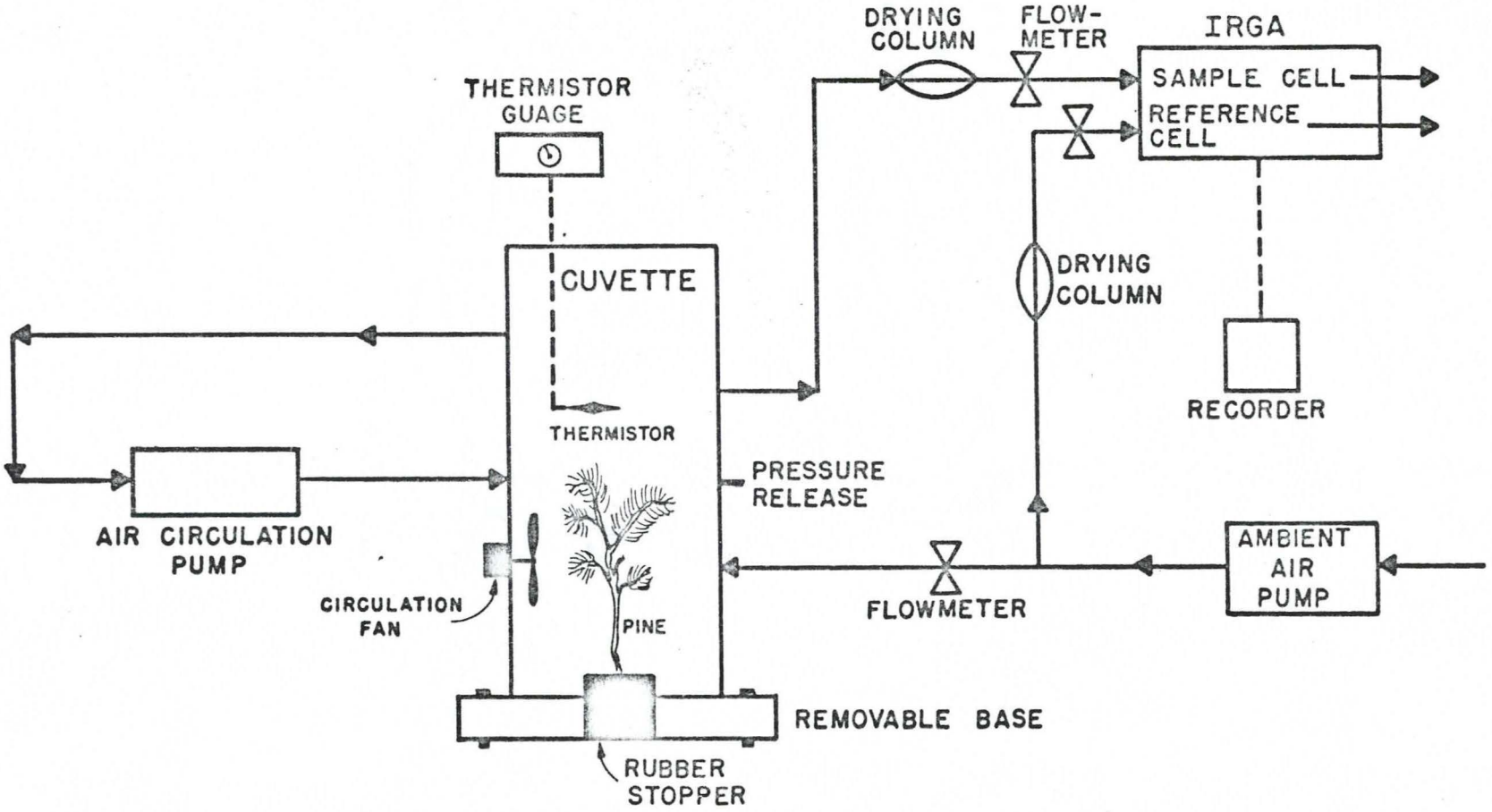


Figure 4. Schematic diagram of open CO_2 gas exchange system used for measuring photosynthetic and respiratory rates for Salt Toxicity Test 2.

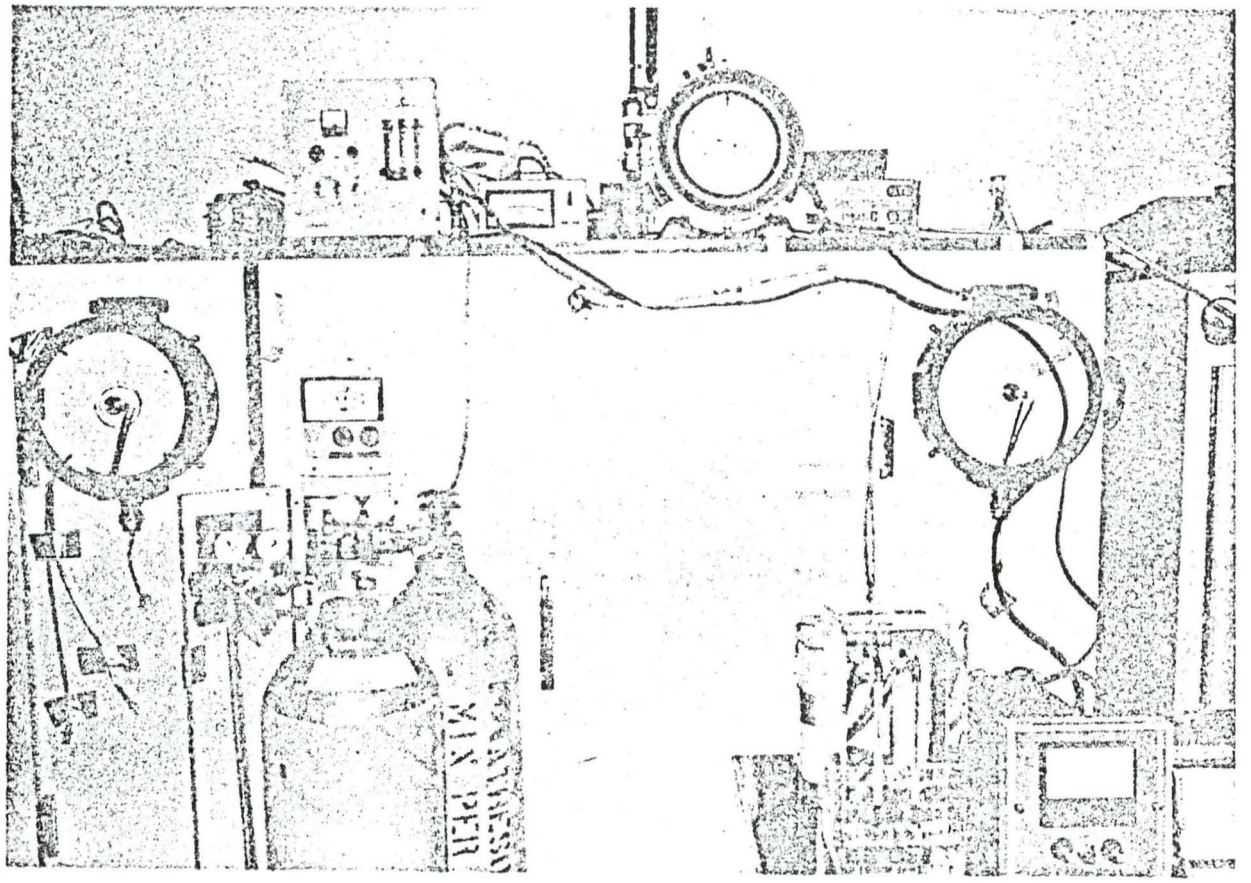


Figure 5. Instrumentation used for the open system of CO_2 gas exchange to measure photosynthesis and respiration rates of ponderosa pine (Pinus ponderosa).

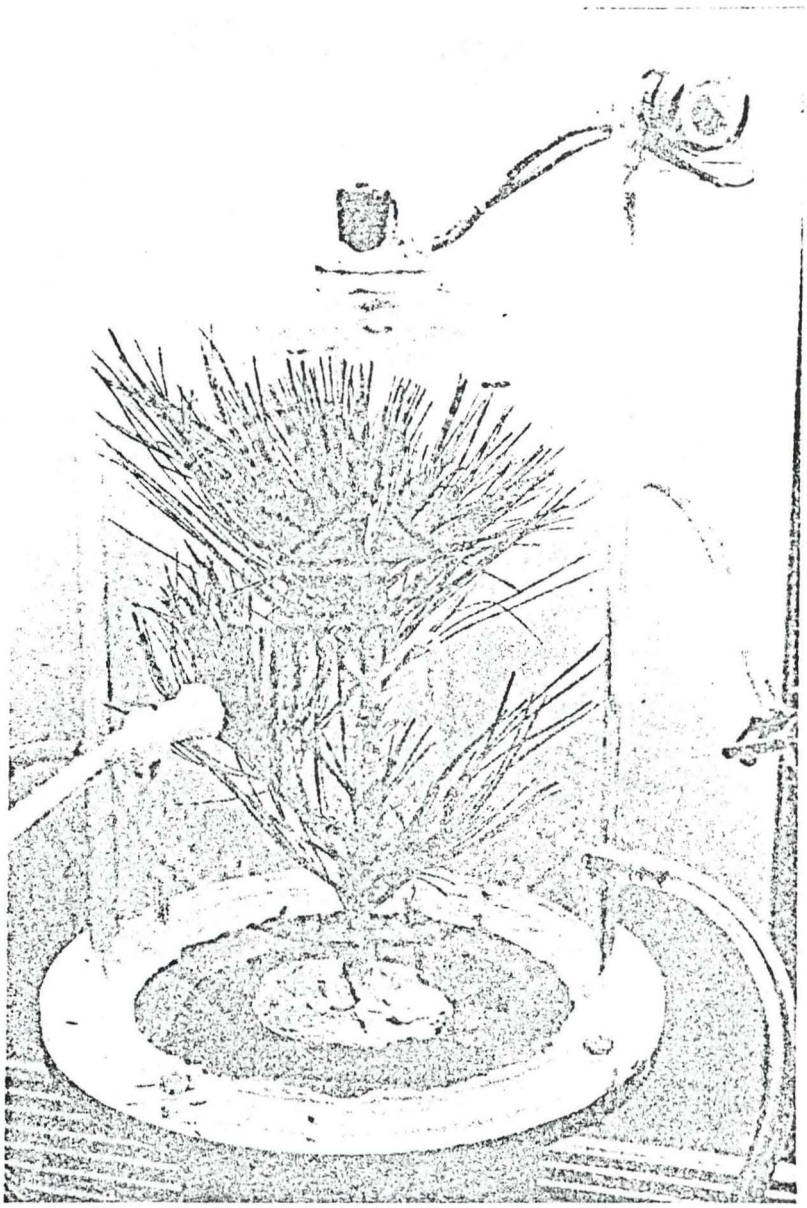


Figure 6. Cuvette used with the open CO_2 gas exchange system for measuring photosynthetic and respiratory rates of ponderosa pine (Pinus ponderosa).

Ambient air was pumped into the system from the atmosphere at approximately 10 m above the soil surface. A subsample of this air was pulled through a 300-liter reservoir then routed through the reference cell of the IRGA for the determination of a base line (zero differential) carbon dioxide concentration. The remainder of the air, 8 to 25 liters·min^{-1}, was routed to the cuvette. Air flow rates were adjusted so that the CO_2 concentration in the cuvette was within $\pm$ 10 percent of the ambient CO_2 concentration. An air sample was withdrawn from the cuvette at a lesser flow rate to insure only outward air leakage from the system. The sample from the cuvette passed through the sample cell of the IRGA. The IRGA, therefore, measured the differential CO_2 concentration (Δppm) between ambient and cuvette CO_2 concentrations. This change in ppm CO_2, along with the flow rate of ambient air entering the cuvette, was used to calculate the CO_2 exchange rate of each pine seedling.

Calculation of CO_2 exchange rates were similar to those of the closed system with the exception that the ambient air flow rate (F) replaced the known volume (V) of the closed system. Net photosynthesis or aboveground dark respiration rates were calculated by the following equation:

$$Pn \text{ or } AGR = (MF/L)\ (\Delta ppm \times 10^{-6}) \ / \ DWG \text{ or } DWT$$

where: M = mole weight of CO_2 (44,010 mg)

F = ambient air flow rate into the cuvette (liters·h^{-1})

L = mole volume of CO_2 (22.414 liters)

No temperature or pressure corrections were necessary in the calculation of CO_2 exchange rates because the flow rates were automatically corrected for temperature and pressure by the mass flow meter used.

Problems with the open gas exchange system were associated with controlling the environmental factors which influence the rates of photosynthesis and respiration. Temperature, humidity and light were held constant by the growth chamber. Light produced by the growth chamber was of a low irradiance (0.27 ly $\cdot$ min^{-1}) from incadescent and fluorescent bulbs and could not be increased because of insufficient cooling ability of the growth chamber. A solution to this problem would be a cooling system associated only with the cuvette. This cooling system could be used to cool the air that is circulated through the cuvette. Designing and building such a cooling system was too expensive to consider for this research. Low irradiance was not considered a problem because the experiment was designed to compare plants growing under equal conditions receiving different osmotic solutions. At light levels used during the experiment, temperature could be held constant by the growth chamber cooling system.

Another problem associated with the system was the possible fluctuations of CO_2 levels in ambient air. This problem was decreased or eliminated by pumping air into the system from 10 m above the soil surface and then routing this air through a 300-liter reservoir. Thus, any changes in CO_2 concentrations would be very slight.

Electrical power fluctuations also caused changes in flow rates and spikes or deflections in recording instruments. It was usually easy to detect the spikes caused by power fluctuations and flow meters were adjusted often to insure accurate flow rates.

Experimental Procedure

Salt Toxicity Test 2 was a split plot factorial design, with CO_2 exchange measurements randomized within block, using six replicates (seedlings) for

each treatment. Differences in CO_2 exchange rates between treatments and dates were compared. Each ponderosa pine seedling was irrigated with 150 ml of a treatment or control solution. The control solution was a 0.25x Hoagland's solution made with distilled water. Treatment solutions were made by adding NaCl or polyethylene glycol 4000 to the control solution until a certain osmotic potential was reached. Thus all plants received the same amount of irrigation treatment and approximately the same amount of nutrients, but certain seedlings received a solution with a lower solute potential (Table 2). The PEG solutions were prepared to be of an equivalent osmotic potential as the NaCl solutions by using unpublished data of J. T. Fisher and C. P. P. Reid (from Mexal and Reid, 1973) showing the relationship between percent PEG 4000 in water (w/v) and its water potential in bars. The osmotic potentials of the NaCl solutions were found in U.S. Agriculture Handbook #60 (Richards, 1954). The osmotic potential of the control was greater than -1.0 bars. The osmotic potential of treated plants on day 1 through day 5 were -1.5, -2.9, -4.4, -5.8, and -7.1 bars, respectively. These osmotic potentials are equivalent to 2000, 4000, 6000, 8000, and 10,000 ppm of sodium chloride.

Two seedlings received foliar spray treatments (Table 2). One foliar spray treatment was distilled water while the other treatment consisted of an NaCl solution. The NaCl solution was made with distilled water and was increased in concentration from 2000 ppm on day 1 to 10,000 ppm on day 5 in increments of 2000 ppm daily. Seedlings receiving the foliar spray treatments were irrigated at the same time with a 0.25x Hoagland's solution.

Table 2. Treatment schedule for Salt Toxicity Test 2.

Day	Control[1]	NaCl irrigation[1]		PEG irrigation[1]
		ppm	Solute potential (bars)	Solute potential (bars)
1	0.25x Hoagland's nutrient solution	2000	-1.47	-1.47
2	0.25x Hoagland's nutrient solution	4000	-2.94	-2.94
3	0.25x Hoagland's nutrient solution	6000	-4.36	-4.36
4	0.25x Hoagland's nutrient solution	8000	-5.77	-5.77
5	0.25x Hoagland's nutrient solution	10000	-7.09	-7.09

	Foliar Spray Treatments	
	Distilled H_2O Spray[2]	NaCl Spray (ppm)[2]
1	Distilled H_2O	2000
2	Distilled H_2O	4000
3	Distilled H_2O	6000
4	Distilled H_2O	8000
5	Distilled H_2O	10000

[1]All treatment solutions (150 ml) were prepared with 0.25x Hoagland's nutrient solution.

[2]Spray treatments also received an irrigation of 150 ml of the 0.25x Hoagland's nutrient solution.

Carbon dioxide exchange was measured 24 h after each plant had received a foliar spray or osmotic solution irrigation treatment. At the completion of each day's gas exchange measurement, the sand culture solution was changed to the next higher concentration of NaCl or PEG. The changing of the sand culture solution was accomplished by using a Millipore vacuum-pressure pump and a Buchner funnel (Figure 7). The changing of the solution was achieved in three steps: (1) most of the sand culture solution was removed by vacuum for four minutes; (2) 150 ml of the next days irrigation treatment was added to the sand culture and most removed by vacuum; (3) another 150 ml of the irrigation treatment was added to the sand culture.

The process of changing the sand culture solution was to insure the same amount of solution in every container and to insure the correct concentration of PEG and NaCl.

Xylem Pressure Potential Measurements

The xylem pressure potential of leaves was measured with a pressure bomb on some seedlings to correlate the effect of treatment osmotic potentials with plant water stress. The procedure for determining the xylem pressure potential of a leaf or stem with the pressure chamber has been outlined by many authors (Scholander et al., 1965; Waring and Cleary, 1967; Barrs, 1968; Boyer, 1969; Zimmerman and Brown, 1971; Ritchie and Hinckley, 1975). The procedure utilized in preparing conifer needles for xylem pressure potential measurements was suggested by Johnson and Nielson (1969). The fascicle sheath was stripped from the pine needles and the xylem traces were cut crosswise to provide a smooth viewing surface. The fascicle was inserted into a rubber

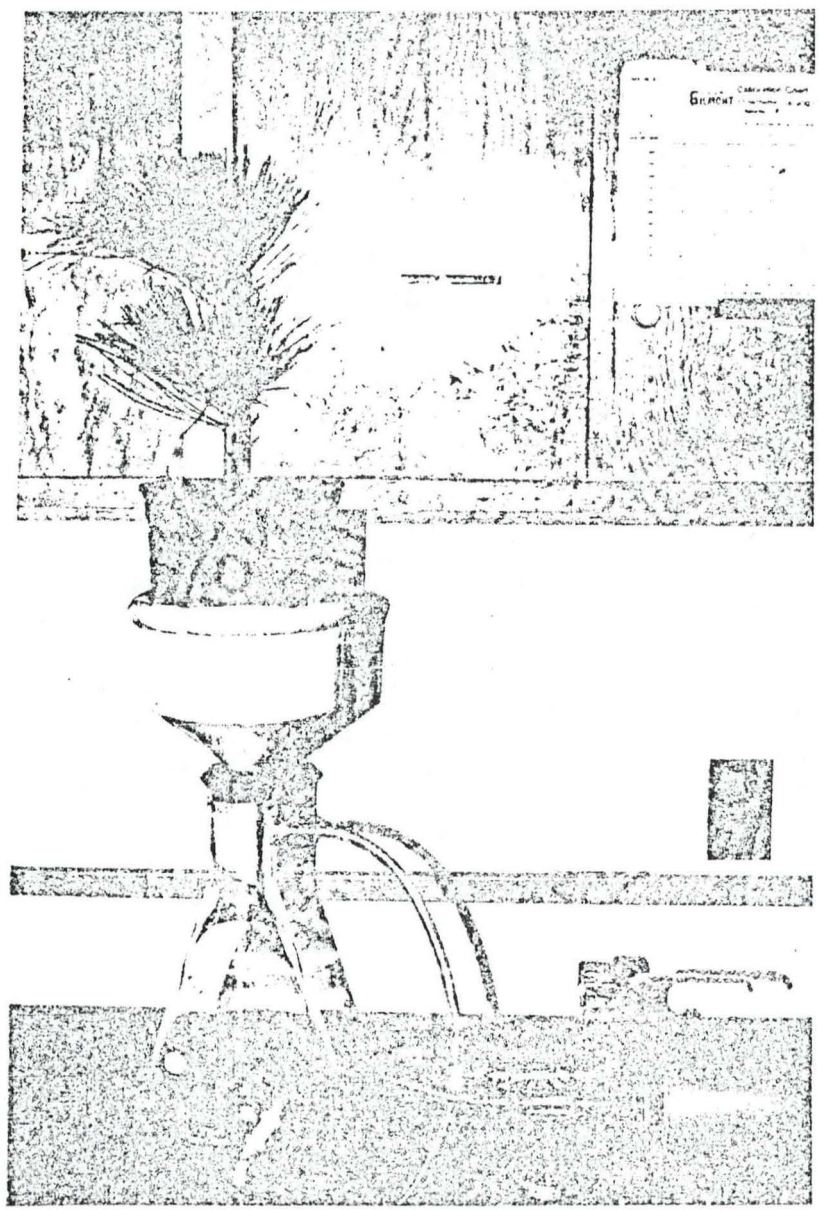


Figure 7. Method of changing irrigation solutions of sand cultures using a Buchner funnel and a millipore-vacuum-pressure pump.

stopper and placed into the pressure chamber. Air pressure was then applied to the chamber until the instant water appeared at the cut end of the xylem traces. A dissecting scope was mounted on top of the pressure chamber to detect the instant water appeared at the xylem traces. When the needle bases were not damaged resin exudation was minimal and endpoints were readily distinguishable. The balancing pressure was then read and recorded. Balancing air pressure was converted to bars by dividing the pounds per square inch (psi) value by 14.5.

Leaf Resistance and Conductance Measurements

Leaf resistance was measured on some seedlings to detect if NaCl foliar sprays, PEG irrigations or NaCl irrigations caused greater stomatal resistance. Stomatal control was measured by leaf resistance or its reciprocal, leaf conductance. Leaf resistance includes cuticular boundary-layer, mesophyll, and stomatal resistances to water vapor diffusion. Most short-term variation in leaf resistance of conifers, however, results from rapid changes in stomatal resistance (Slatyer, 1967; Waggoner and Turner, 1971). Leaf resistance was measured directly with a null-balance diffusion porometer on intact foliage and leaf conductance was calculated as the leaf resistance reciprocal according to the procedure of Running (1976). Leaf area was measured by using an optical planimetry method in which surface area was calculated from length and diameter measurements (Drew and Running, 1975).

Foliar Measurements

The concentrations of sodium and chloride in leaf materials was measured on all seedlings in both salt toxicity tests. In Salt

Toxicity Test 1, foliar injury measurements were recorded. There was no apparent foliar browning in Salt Toxicity Test 2. Therefore, no foliar injury measurements were recorded.

Determination of Sodium and Chloride Levels

All leaf material was dried at 60°C and then ground to pass through a 40-mesh screen. Levels of foliar chloride were determined by a potentiometric titration using a Beckman #476126 specific ion electrode and a Sargent-Welch recording titrator model DG. The potentiometric titration, using a chloride-specific ion electrode as an end point indicator, was recommended by Dr. Leon Bernstein of the U.S. Salinity Laboratory, Riverside, California, as being most versatile and accurate (from Button, 1971). The procedure followed in these determinations was that recommended by Button (1971).

Levels of foliar sodium were determined flame photometrically according to the procedure recommended by the Association of Official Agricultural Chemists. A weighed foliage sample was transferred to a 120 ml Ehrlenmeyer flask and 50 ml of 0.24N ammonium oxalate solution was added as an extracting solution. The Ehrlenmeyer flask was stoppered and mechanically shaken for one hour. The solution was filtered through dry fast filter paper and the filtrate used for determinations. A Beckman DU-2 Spectrophotometer was used for all measurements. Sodium standards spanning the concentration range of the unknown samples were run at frequent intervals during atomization of a series of samples. This operation was repeated with both standard solutions and sample solutions enough times to result in reliable average readings for each solution. Standard curves were plotted from readings of the sodium

standards and were used in calculating the percentage of sodium in the samples.

Foliar Injury Measurements

The amount of foliar injury for each seedling in Salt Toxicity Test 1 was estimated on February 12 and April 1, 1976. Estimates of foliar injury consisted of grouping trees into broad classes based on the visual percentage of foliar browning.

Quantitative measurements of foliar injury were recorded on May 31 and June 25, 1976. On May 31, an occular estimate of the percentage of foliar browning on a leaf area basis was made. The procedure on May 31 was to measure the average width of needles. This value was multiplied by the number of fascicles per tree times the average number of needles per fascicle times the average length of needles. On June 25 the percentage of foliar browning for each seedling on a weight basis was determined. Needles from each seedling were hand separated into green and brown portions, dried at 60°C and then weighed.

Soil Measurements

Soil samples were analyzed for electrical conductivity (EC_e), pH, and levels of sodium and chloride. All soil analyses from Salt Toxicity Test 1 were performed on filtrate solutions from saturated soil paste. The extracts were prepared by saturating a 250 g soil sample from each pot with distilled water, allowing 1 h for reactions to occur and then filtering the solution from the soil paste through Whatman No. 44 filter paper under vacuum (Richards, 1954). Prior to any treatments the soil mixture used in the experiment was found to have an average EC_e value of 0.6 mmhos/cm x 10^3 (18 ppm of sodium and 36 ppm of chloride).

For Salt Toxicity Test 2 the sand culture containers were put under direct vacuum and the filtrate was used for measurements of EC_e, pH, and levels of sodium and chloride.

Electrical Conductivity and pH Measurements

The electrical conductivity was used for indicating the total concentration of the ionized constituents of the soil extracts. This value is closely related to the sum of the cations (or anions) as determined chemically and usually correlates closely with the total dissolved solids (Richards, 1954). The electrical conductivity of the saturated soil extracts was measured with a RD 26 Solu-Bridge.

Soil pH was measured using a Sargent-Welch pH electrode #S-30072-15 and an Orion model 801A digital ionalyzer pH/mv meter.

Sodium and Chloride Levels

Soil sodium and chloride levels were determined by direct measurement of the filtrate solutions of soil extracts, using Orion specific ion electrodes #96-11 and #96-17, respectively, and an Orion digital ionalyzer pH/mv meter model 801A. The procedures involved in these measurements were outlined in the instruction manuals for use of the electrodes. Many researchers have found direct measurements using specific ion electrodes for sodium and chloride to be correlated with chemical methods but simpler and less time consuming to use (Hipp and Landale, 1971; Wood, 1973; Smart, Thomas and Drover, 1974).

Statistical Analyses

Statistical analyses of data were performed using a CDC 6400 computer on the Colorado State University campus. The Statistical Package for the Social Science (SPSS) statistical programs were utilized. Analyses

of variance and Duncan's test were used to test at the 0.05 level of probability unless otherwise specified. Analyses of covariance was used to test the effects of soil sodium and chloride levels on CO_2 exchange rates of ponderosa pine seedlings in Salt Toxicity Test 1.

RESULTS AND DISCUSSION

Results of Salt Toxicity Test 1 and Salt Toxicity Test 2 are presented in separate sections. Salt Toxicity Test 1 is further subdivided into three separate discussions. The first discussion of Salt Toxicity Test 1 is concerned with the foliar injury of ponderosa pine seedlings resulting from sodium chloride foliar spray and sodium chloride irrigation treatments. The second discussion is about the effects of sodium chloride irrigation and foliar spray treatments on different soil and plant variables. Results of sodium chloride foliar sprays on net photosynthesis and dark respiration rates is presented in the third discussion. The effects of different osmotic potential solutions used in Salt Toxicity Test 2 on some soil and plant variables is discussed in the final section.

Salt Toxicity Test 1

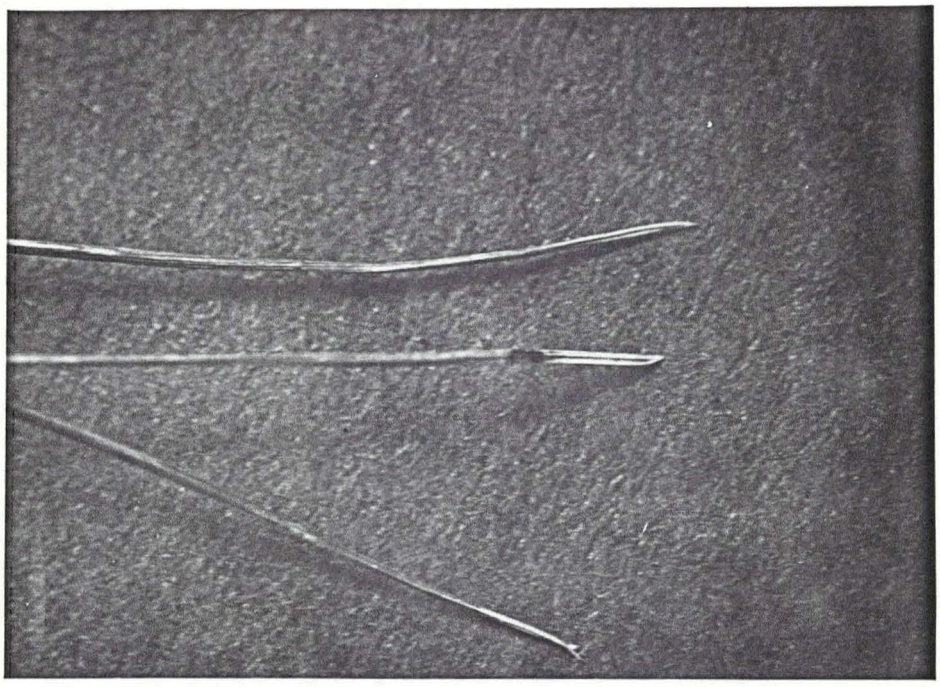
Foliar Injury

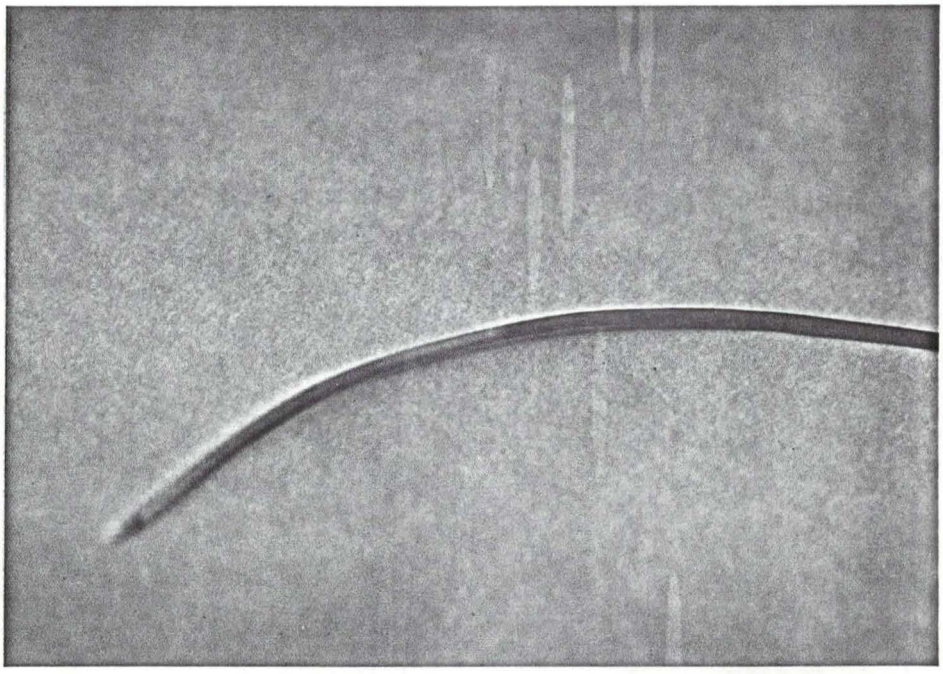
Foliar browning of the tip of ponderosa pine needles (leaves) was observed within five weeks after treatments with sodium chloride (NaCl) began. Injury appeared most often at the tip of pine needles (Fig. 8), but also started from the center of the needle (Fig. 9). A distinct band usually appeared within 12 mm of the tip of the injured needle. As injury increased, tissue distal to the band became mottled and necrotic. Spotts (1969) described this band as a resin infiltration produced by chloride salts.

The main effect of NaCl irrigations and foliar sprays on foliar injury levels was significant at all four dates in which injury estimates were recorded (Appendix, Table 1). Foliar spray caused low

Figure 8. Ponderosa pine (*Pinus ponderosa*) needles (leaves) treated with NaCl showing tip burn and banding.

Figure 9. Ponderosa pine (*Pinus ponderosa*) treated with NaCl showing injury to the mid portion of the needle.





injury levels, whereas salt irrigations soon became fatal to almost all plants.

Foliar injury developed earlier and to a larger extent on plants that received foliar spray treatments than for non-sprayed plants (Table 3). At the time of the first injury estimate (February 12, 1976), the effects of foliar spray and irrigation treatments were evident (Appendix, Table 1). Plants had received eight treatments by this date. There was also a significant interaction between foliar spray and irrigation treatments. Plants that received distilled water irrigations and the 6000 or 9000 ppm NaCl foliar spray had injury levels of five to six percent and were similar to plants receiving the 9000 ppm irrigations and the 6000 and 9000 ppm foliar spray treatments (Table 3). Plants in the 3000 and 6000 ppm NaCl irrigation treatments had low injury levels of two to four percent at every level of foliar spray. The average injury level for plants in the 9000 foliar spray treatment were as high or higher than injury caused by other foliar spray treatments (Table 3).

On April 1, 1976, foliar injury levels of ponderosa pine seedlings had increased for almost every foliar spray and irrigation treatment (Table 4). The seedlings had been treated 13 times with NaCl by this date. The effect of irrigation treatments had become significant at the 0.01 level of probability, whereas the foliar spray treatment effects were significant at the 0.10 level of probability (Appendix, Table 1). There was a general trend of increasing injury with increasing concentration of the NaCl foliar spray. However, plants irrigated with 6000 and 9000 ppm NaCl showed no significant trend of increasing injury with increasing concentration of foliar spray. By this date, plants

Table 3. Average foliar injury (%) of ponderosa pine (Pinus ponderosa) on February 12, 1976, as affected by sodium chloride foliar spray and irrigation treatments.

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
0	2.0a[1]	2.0a	2.0a	2.0a
3000	2.0a	2.0a	2.0a	4.0abc
6000	4.8abc	2.6ab	2.0a	5.0bc
9000	5.8c	2.6ab	2.6ab	4.2abc

[1]Means in any row or column followed by a similar letter are not significantly different at the 0.05 level of probability.

Table 4. Average foliar injury (%) of ponderosa pine (Pinus ponderosa) on April 1, 1976, as affected by sodium chloride foliar spray and irrigation treatments.

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
0	2.0a[1]	3.6a	55.8bcd	93.4e
3000	2.0a	19.4a	26.0ab	73.8de
6000	7.8a	21.0a	60.8cd	54.6bcd
9000	12.8a	31.0abc	65.6de	92.6e

[1]Means followed in any row or column by a similar letter are not significantly different at the 0.05 level of probability.

in the 6000 and 9000 ppm NaCl irrigation treatments had average injury levels of 53 and 79 percent, respectively. These were significantly greater injury levels than for plants receiving the distilled water irrigation or the 3000 ppm irrigation, except those receiving a combination of 9000 ppm NaCl foliar spray and 3000 ppm NaCl irrigation. Injury levels for the distilled water and 3000 ppm NaCl irrigation averaged six and 19 percent, respectively.

By May 31, 1976, there was no longer any significant increase in foliar injury attributed to NaCl foliar spray treatments, but irrigation treatments significantly increased injury levels (Appendix, Table 1). Both the 6000 and 9000 ppm NaCl irrigated plants had significantly higher injury levels than did plants receiving the other irrigation treatments (Table 5). Almost total foliar browning had occurred for plants receiving the 9000 ppm NaCl irrigation. Injury level for plants receiving the 6000 ppm irrigation approached 90 percent. The 3000 ppm irrigation treatment had also caused significantly higher injury levels than for plants that received the distilled water irrigation (Table 5).

Total death loss of plants receiving the 6000 and 9000 ppm NaCl irrigations had occurred by June 25, 1976. Plants receiving the 3000 ppm irrigations had average injury levels of about 84 percent which were not statistically different from the 6000 or 9000 ppm irrigation treatments (Table 6). All plants that received sodium chloride irrigation treatments had significantly higher injury levels than did plants receiving distilled water irrigations. Foliar spray treatments did not cause any increased foliar injury (Appendix, Table 1).

Table 5. Average foliar injury (%) of ponderosa pine (Pinus ponderosa) on May 31, 1976, as affected by sodium chloride foliar spray and irrigation treatments.

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H2O	3000 ppm	6000 ppm	9000 ppm
0	2.6a[1]	37.2b	90.8e	99.0e
3000	2.6a	56.2bc	66.6cd	99.0e
6000	8.8a	57.4bc	90.0e	99.0e
9000	13.2a	60.8c	84.8de	99.0e

[1]Means in any row or column followed by a similar letter are not significantly different at the the 0.05 level of probability.

Table 6. Average foliar injury (%) of ponderosa pine (Pinus ponderosa) on June 25, 1976, as affected by sodium chloride foliar spray and irrigation treatments.

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
0	5.6a[1]	73.0b	100c	100c
3000	6.0a	90.4bc	100c	100c
6000	13.8a	87.8bc	100c	100c
9000	13.0a	87.8bc	100c	100c

[1]Means in any row or column followed by a similar letter are not significantly different at the 0.05 level of probability.

All sodium chloride treatments were discontinued March 18, 1976, before the second injury estimate. There was a trend of increasing injury caused by foliar spray for the second injury estimate. However, at the last two dates in which injury estimates were recorded there was no significant trend in injury attributable to the previous foliar spray treatments. Since much of the foliar-applied sodium chloride was probably washed from the leaves by snow and rain, there probably should not have been increasing injury at the last two dates caused by earlier additions of foliar spray.

It becomes evident from the preceeding discussion that the NaCl irrigation treatments were the primary causative agent for foliar injury. Small amounts of foliar injury occurred quickly from sodium chloride foliar spray, but did not increase significantly through time. The rapid toxic reaction from foliar spray probably resulted from direct foliar absorption of the sodium and cloride ions. Wittwer and Teubner (1959) have shown that foliar absorption of sodium and chloride does occur.

Other Plant and Soil Variables

Sodium chloride irrigations significantly affected soil and foliar chloride levels, soil and foliar sodium levels and electrical conductivity (EC_e) of the soil (Appendix, Tables 1 and 2). The two-way interactive effects of sodium chloride foliar spray and irrigation also significantly affected these plant and soil variables. However, the effect of foliar spray alone only significantly affected foliar chloride and sodium levels.

Interactions between foliar spray and irrigation treatments occurred because of an inconsistent effect of foliar sprays on different plant and soil variables. For the 3000 and 6000 ppm sodium chloride irrigations, the seedlings that also received the 3000 ppm NaCl foliar spray treatment had slightly greater increases of soil sodium (Table 7), soil chloride (Table 8), and EC_e (Table 9), than did plants receiving foliar sprays of distilled water, 6000 ppm, or 9000 ppm sodium chloride. Under the 9000 ppm sodium chloride irrigation treatment, the level of soil sodium and chloride, EC_e and foliar chloride and foliar sodium levels were higher for treatments receiving the distilled water spray and lowest for treatments receiving a foliar spray of 3000 ppm sodium chloride (Tables 7 through 11).

Ponderosa pine seedlings that received the 3000 ppm sodium chloride irrigations had average levels for electrical conductivity in the soil of 6 mmhos/cm compared to an average value of 1.1 mmhos/cm for seedlings that received distilled water irrigations (Table 9). Electrical conductivity was as high as 17 mmhos/cm for the 9000 ppm irrigation treatment. The EC_e of a soil can be multiplied by -0.365 bars to give the approximate solute potential of the soil (Richard, 1954). A soil with an EC_e of 17 mmhos/cm would have a soil solute potential of about -6.2 bars, which could be critically high to plant water status as the soil dried.

Merkhoven et al. (1965) found that an EC_e value of about 6 mmhos/cm was critical to the survival of Scots pine (Pinus sylvestris). Only a few plants could survive in soils with as high conductivity as that found in the 9000 ppm sodium chloride irrigation treatment.

Table 7. Effects of sodium chloride foliar spray and irrigation treatments on levels of sodium (ppm) in the soil.

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
0	14a[1]	668b	1616c	2890f
3000	35a	986b	2002cd	2187d
6000	25a	808b	1662c	2404de
9000	26a	735b	1648c	2710ef

[1]Means in any row or column followed by a similar letter are not significantly different at the 0.05 level of probability.

Table 8. Effects of sodium chloride foliar spray and irrigation treatments on chloride levels (ppm) in the soil.

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
0	41a[1]	1095b	2005de	3192h
3000	101a	1338bc	2378ef	2391ef
6000	83a	1163b	1821cd	2629fg
9000	66a	1018b	1908de	2912gh

[1]Means in any row or column followed by a similar letter are not significantly different at the 0.05 level of probability.

Table 9. Effects of sodium chloride foliar spray and irrigation treatments on electrical conductivity (mmhos · cm^{-1} at 25°C) in the soil.

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
0	1.1a[1]	5.8b	10.3de	17.0h
3000	1.1a	7.6bc	12.2def	12.8ef
6000	1.1a	6.0b	9.7cd	14.0fg
9000	1.0a	5.8b	10.1cd	15.6gh

[1]Means in any row or column followed by a similar letter are not significantly different at the 0.05 level of probability.

Table 10. Effects of sodium chloride foliar spray and irrigation treatments on the percentage of chloride in foliage of ponderosa pine (Pinus ponderosa).

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
0	0.043a[1]	1.356b	2.181d	3.519e
3000	0.066a	1.430b	2.231d	2.050cd
6000	0.096a	1.521b	2.255d	3.341e
9000	0.102a	1.669bc	2.028cd	3.486e

[1]Means in any row or column followed by a similar letter are not significantly different at the 0.05 level of probability.

Table 11. Effects of sodium chloride foliar spray and irrigation treatments on the percentage of sodium in foliage of ponderosa pine (Pinus ponderosa).

Foliar spray (NaCl ppm)	Irrigation (NaCl)			
	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
0	0.009a[1]	0.593b	0.766b	1.886g
3000	0.046a	0.637b	1.104cde	1.368def
6000	0.072a	0.648b	1.250e	1.615fg
9000	0.078a	0.850bc	1.059cd	1.806g

[1]Means in any row or column followed by a similar letter are not significantly different at the 0.05 level of probability.

Levels of sodium and chloride in the foliage of plants subjected to sodium chloride irrigations were also at or near toxic levels. Mean foliar chloride levels were above 1.4 percent in plants subjected to sodium chloride irrigations compared to less than 0.1 percent for seedlings receiving the distilled water irrigations. Many researchers have found foliar chloride levels of 1.0 percent or greater to be toxic to plants (Ehlig, 1964; Holmes and Baker, 1966; Westing, 1969). Sodium levels in foliage were above 0.6 percent for all sodium chloride irrigated plants compared with average values of less than 0.08 percent for seedlings receiving distilled water irrigations. Smith (1970) found high levels of sodium in foliage samples of injured white pine along the Connecticut Turnpike. High sodium concentrations might also increase the permeability of cell membranes (Heimann, 1966).

Therefore, sodium chloride irrigation treatments probably caused foliar injury by increasing the osmotic potential of the soil water and creating plant water stress, or by causing increased sodium and chloride ions in the foliage to levels that were toxic, or by a combination of water stress and specific ion toxicity.

Net Photosynthesis and Dark Respiration Rates

The main treatment effects of sodium chloride foliar sprays on both net photosynthesis (green leaf material) and dark respiration rates of ponderosa pine seedlings were nonsignificant (Appendix, Table 3). However, a general trend of decreasing net photosynthesis with increasing NaCl concentration was evident for plants from all the sodium chloride foliar spray treatments (Table 12). Photosynthetic rates declined from about 3.2 mg $CO_2 \cdot g^{-1} \cdot h^{-1}$ for control plants to about 2.6 mg $CO_2 \cdot$

Table 12. Average net photosynthesis (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$) of ponderosa pine (Pinus ponderosa) as affected by sodium chloride foliar spray.

Net photosynthesis based on green & total weight	Spray treatments (NaCl)				
	Control	Distilled H_2O	3000 ppm	6000 ppm	9000 ppm
Green leaf dry weight	3.20a[1]	2.95a	2.42a	2.63a	2.81a
Total leaf dry weight	3.14a	2.85ab	2.30b	2.36ab	2.59ab

[1]Means in the same row followed by a similar letter are not significantly different at the 0.10 level of probability.

$g^{-1} \cdot h^{-1}$ for plants that received high concentrations of NaCl foliar spray. Since concentrations of sodium and chloride could not be maintained at a constant level in soil within the pots because of sodium chloride loss from needles to the soil, a covariance analysis was used to test effects of the soil sodium and chloride levels on CO_2 exchange rates. Soil sodium and chloride concentrations were found to have a significant effect on net photosynthesis, but did not significantly affect dark respiration rates (Appendix, Tables 4 and 5). A simple linear regression showed that as soil chloride and sodium increased, net photosynthesis significantly decreased (r^2 = 0.31 and 0.29, respectively.

The foliar spray treatments significantly increased levels of soil chloride and sodium (Appendix, Table 6). The concentration of both soil sodium and chloride was highest for the 3000 ppm sodium chloride foliar spray treatment, although they were not significantly different from the 6000 or 9000 ppm sodium chloride foliar spray (Fig. 10). Sodium and chloride levels in the soil from the 6000 and 9000 ppm foliar spray treatments were not significantly different from either the control or distilled water spray treatments, but the average concentrations of soil sodium and chloride were almost twice as high.

The reason for a higher concentration of soil sodium and chloride in the lowest sodium chloride foliar spray treatment is not known. The possibility of using the wrong concentration of foliar spray on these seedlings is unlikely because it was the same solution that was used for the 3000 ppm irrigation treatment. Soil sodium and chloride levels progressively increased from the 3000 sodium chloride irrigation

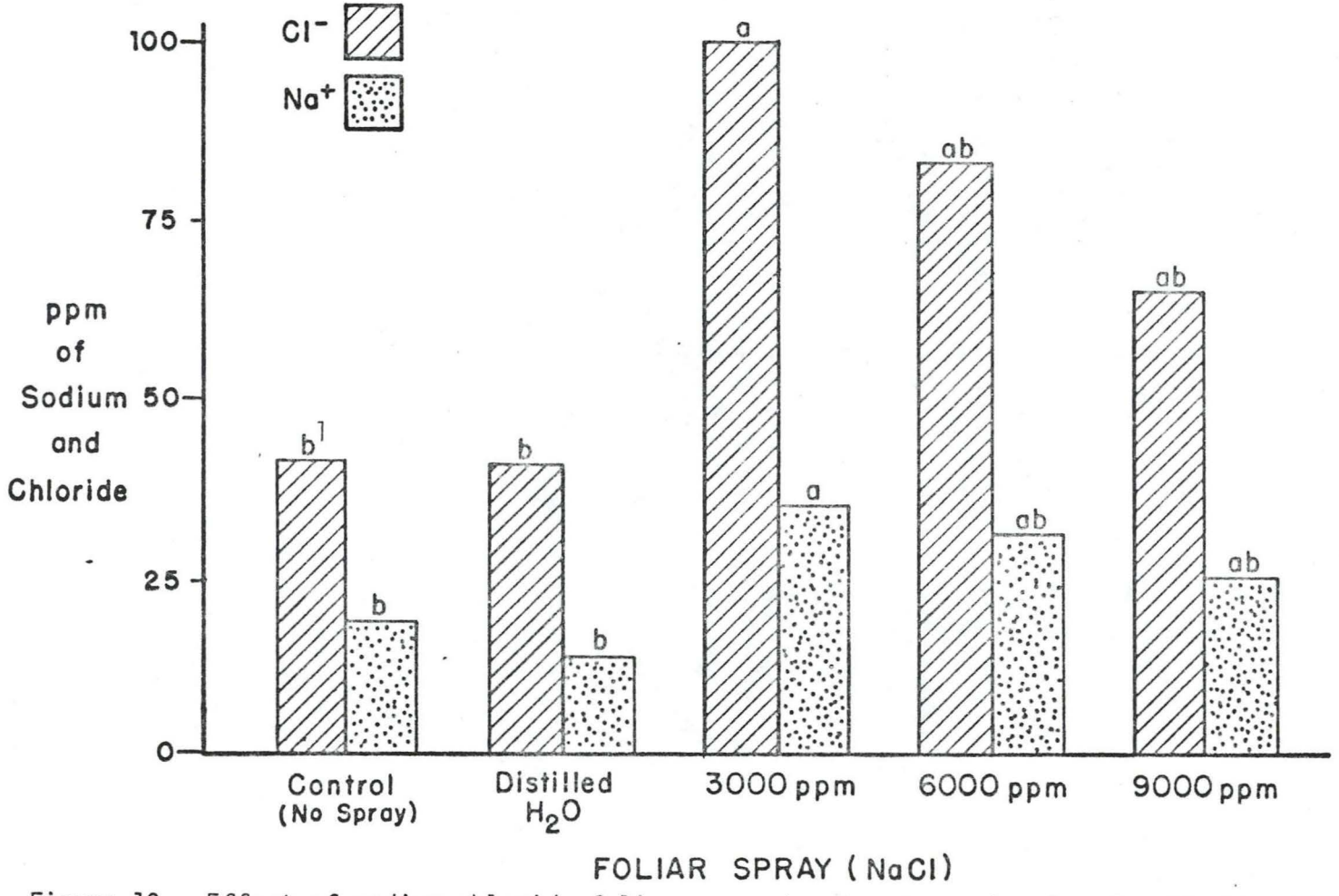


Figure 10. Effect of sodium chloride foliar spray treatments on levels of soil chloride and soil sodium.

[1]Means of the same ion with a similar letter are not significantly different at the 0.05 level.

to the 9000 ppm irrigation (Tables 7 and 8). Levels of sodium and chloride in the soil did not parallel levels of sodium and chloride in the foliage. This may have resulted from needles not being equally rinsed by natural precipitation for the complete removal of sodium chloride adhering to foliage or from unequal drip from the foliage.

Thus, from the analyses of covariance it is evident that soil chloride and sodium concentrations caused by foliar sprays did affect net photosynthetic rates. Soil levels of these ions were, however, too low in concentration to decrease soil water osmotic potentials, and were probably too low to cause nutritional problems. Therefore, it is postulated that the effects of the soil sodium and chloride concentrations on photosynthetic rates of the ponderosa pine seedlings was probably caused by a specific ion effect. The decrease in photosynthesis could be a result of the effect of NaCl on the chloroplast. Lapina and Bikmukhametova (from Campbell and Pitman, 1971) found that the Hill reaction activity was depressed in chloroplasts of corn plants (Zea mays) receiving NaCl treatments.

Salt Toxicity Test 2

Ponderosa pine seedlings were grown under controlled conditions in sand cultures prior to treatments in Salt Toxicity Test 2. Statistical analysis of CO_2 exchange rates (net photosynthesis and dark respiration) for Salt Toxicity Test 2 were based on differences between the CO_2 exchange rates of Day 2 through Day 5 minus the rates of CO_2 exchange of Day 1. This was done to reduce variability caused by large differences in CO_2 exchange rates among seedlings. The CO_2 exchange rates on Day 1 were usually the highest and decreased with time. Therefore, data values usually became more negative with increasing time.

The CO_2 exchange rates of ponderosa pine seedlings were not significantly affected by irrigations with solutions of different osmotic potentials (Appendix, Table 7). Large differences among replications may have been the reason for not finding a significant effect on net photosynthetic rates caused by the various treatments (Appendix, Table 7). There were also no significant differences in CO_2 exchange rates of seedlings receiving distilled water or sodium chloride foliar sprays. A trend of decreasing net photosynthetic rates with irrigations using solutions of lower osmotic potentials was, however, evident (Table 13). The average change in net photosynthetic rates for the different treatments were -0.76 for the control plants, -1.97 for seedlings receiving NaCl foliar spray, -2.01 for plants that received distilled water spray, -2.62 for seedlings irrigated with PEG, and NaCl-irrigated plants had the greatest decrease in net photosynthetic rates of -2.87 mg $CO_2 \cdot g^{-1} \cdot h^{-1}$. Dark respiration rates also decreased

Table 13. Adjusted and unadjusted mean values of net photosynthesis (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$) of ponderosa pine (Pinus ponderosa) as affected by solutions of different osmotic potentials.

Time	Treatment									
	Control		NaCl foliar spray		Distilled H_2O spray		PEG irrigation		NaCl irrigation	
Day 1		(6.81)[2]		(5.77)		(5.77)		(5.43)		(5.03)
Day 2	-0.245[1]	(6.57)	-1.525	(5.29)	-1.97	(4.93)	-1.88	(4.93)	-2.39	(4.42)
Day 3	-0.062	(6.75)	-1.598	(5.22)	-0.56	(6.34)	-2.23	(4.58)	-1.92	(4.89)
Day 4	-1.447	(6.86)	-2.077	(6.23)	-3.35	(5.60)	-2.72	(5.58)	-3.23	(5.07)
Day 5	-1.897	(6.41)	-3.067	(5.24)	-3.13	(5.81)	-4.18	(4.12)	-4.63	(3.68)

[1]Values were adjusted by subtracting the first day's net photosynthetic rate for each seedling from all following net photosynthetic rates.

[2]Values in parenthesis are mean values of unadjusted net photosynthetic rates.

with solutions of decreasing osmotic potential (Table 14). The average change in dark respiration rates for the different treatments were -0.10 for control plants, - 0.01 for distilled water sprayed plants, -0.12 for sodium chloride foliar sprayed plants, -0.17 for PEG irrigated plants, and -0.18 mg $CO_2 \cdot g^{-1} \cdot h^{-1}$ for sodium chloride irrigated plants.

Time of data collection (Days) significantly ($p < 0.01$) affected CO_2 exchange rates (Appendix, Table 7). The time effect might have resulted from plants becoming light saturated over the length of the experiment. The inhibition of photosynthesis has been found to result from intense light or exposure to long periods of light. The decline of net photosynthesis with time might have also resulted from accumulation of photosynthetic products in chloroplasts (Kozlowski, 1957; Spence and Humphries, 1972). With low irradiances used in this experiment, however, light saturation may not have been achieved. Inadvertent handling of the plants may have reduced net photosynthetic rates through time.

The time by treatment interaction on net photosynthesis was significant ($p < 0.10$). This interaction probably resulted from greater net photosynthetic rates on Day 3 for the control plants and for ponderosa pine seedlings receiving the distilled water spray and the sodium chloride irrigation treatments (Table 13).

There was a significant treatment effect for all other plant and soil variables measured in this study, except pH (Appendix, Tables 8 and 9). Sodium chloride irrigations significantly increased electrical conductivity, soil sodium and soil chloride levels. Sodium chloride

Table 14. Adjusted and unadjusted mean values of dark respiration (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$) of ponderosa pine (Pinus ponderosa) as affected by solutions of different osmotic potentials.

Time	Treatment									
	Control		NaCl foliar spray		Distilled H_2O spray		PEG irrigation		NaCl irrigation	
Day 1		(1.26)[2]		(1.31)		(1.06)		(1.23)		(1.34)
Day 2	0.15	(1.40)	0.07	(1.32)	0.11	(1.23)	-0.14	(1.11)	0.16	(1.41)
Day 3	-0.20	(1.06)	-0.22	(1.04)	-0.19	(0.98)	-0.14	(1.11)	-0.23	(1.02)
Day 4	-0.40	(0.81)	-0.39	(0.82)	0.06	(1.11)	-0.22	(0.98)	-0.41	(0.79)
Day 5	0.00	(1.21)	0.02	(1.23)	0.04	(1.09)	-0.20	(1.01)	-0.34	(0.87)

[1]Values were adjusted by subtracting the first day's respiration rate for each seedling from all following respiration rates.

[2]Values in parenthesis are mean values of unadjusted respiration rates.

foliar sprays also increased these variables, although differences were not significant (Table 15).

After six days, ponderosa pine seedlings that received sodium chloride irrigations had approximately ten times the concentration of sodium and chloride in the foliage than did plants that did not receive sodium chloride irrigations (Table 16). Sodium chloride applied as a foliar spray caused a significant increase in sodium concentration in the foliage and also greatly increased foliar chloride levels above plants not receiving the sodium chloride spray. However, the sodium chloride irrigated seedlings had much higher levels of sodium and chloride in the foliage than did plants from all the other treatments.

The average xylem water potentials of ponderosa pine seedlings under constant light that received the control irrigation solution (0.25x Hoagland's solution) were between -6.8 and -8.1 bars. Seedlings that received the PEG or sodium chloride irrigation treatments had significantly lower xylem water potentials which averaged -12.3 and -12.2 bars, respectively (Table 16). After the ponderosa pine seedlings received a 24-hour dark period, xylem water potentials were between -1.7 and -2.0 bars for plants receiving the control irrigations, and -7.2 and -7.3 bars for the PEG and NaCl irrigated seedlings, respectively.

Leaf resistances averaged 35 s/cm for the NaCl irrigated plants. Seedlings in other treatments had significantly lower leaf resistances. Plants in the sodium chloride foliar spray treatment had the lowest leaf resistance of about 10 s/cm compared to an average resistance of 13 s/cm for control plants, plants sprayed with distilled water,

Table 15. Average effects for solutions of different osmotic potentials on some soil characteristics.

Treatment	pH	EC_e (mmhos/cm)	Soil Na^+ (ppm)	Soil Cl^- (ppm)
Control	6.3a	0.8a[1]	9a	1a
NaCl spray	6.4a	1.2a	67a	98a
Water spray	6.4a	0.8a	9a	3a
P.E.G. Irrigation	6.5a	0.3a	19a	3a
NaCl Irrigation	6.6a	23.9b	4872b	5780b

[1]Means in the same column followed by a similar letter are not significantly different at the 0.05 level of probability.

Table 16. Average effects for solutions of different osmotic potentials on some plant characteristics.

Treatment	Foliar Na^+ (%)	Foliar Cl^- (%)	XWP[2] Constant light (-bars)	XWP- 24h dark (-bars)	Leaf Resistance (s/cm)	Leaf Conductance (cm/s)
Control	0.012a[1]	0.034a	8.0a	2.0a	12.95a	0.088a
NaCl spray	0.047b	0.073a	8.1a	1.7a	9.97a	0.101a
Water spray	0.014a	0.038a	6.8a	1.7a	13.12a	0.079a
P.E.G. Irrigation	0.011a	0.037a	12.3b	7.2b	13.00a	0.077a
NaCl Irrigation	0.104c	0.320b	12.2b	7.3b	35.00b	0.028a

[1]Means in the same column followed by a similar letter are not significantly different at the 0.05 level of probability.

[2]XWP = Xylem Water Potential.

and plants irrigated with PEG solution (Table 16). The large increase in leaf resistance for ponderosa pine seedlings receiving the sodium chloride irrigation treatments is surprising. Running (1975) found that leaf resistances of ponderosa pine were fairly stable from xylem water potentials of -5 to -15 bars. At approximately -15 bars, leaf resistance increased rapidly. Salisbury and Ross (1969) stated that stomatal aperature was highly variable and thus responsible for most changes in leaf resistance. The stomatal aperature is largely dependent on plant water status, but the potassium cation has also been found to be important in stomatal movement. Fujino (Raschke, 1975) proposed that the migration of potassium into guard cells occurred in quantitites large enough to increase osmotic pressure and cause stomatal opening. Chloride has been found to neutralize some of the positive charges of potassium in the stomata of many plants and chloride accompanies potassium on its way into and out of guard cells (Raschke, 1975). Ponderosa pine seedlings irrigated with sodium chloride and PEG had approximately the same xylem water potentials, but leaf resistances were much higher for plants that received sodium chloride treatments. Since the ponderosa pine seedlings had much higher concentrations of sodium and chloride ions in the foliage, this may have interferred with the potassium balance in the guard cells, resulting in higher stomatal resistances.

Leaf resistance is of special importance because it is the major physiological control of plant water status and directly controls transpiration and often photosynthesis. Average net photosynthetic rates of sodium chloride and PEG-irrigated plants were very similar

and probably reveal similar leaf resistances which is in contrast to actual measured leaf resistance. The reason for this was that leaf resistances and xylem water potential was measured 24 h after the last CO_2 exchange measurement which did not actually correspond to CO_2 exchange measurements. During CO_2 analysis, stomatal closure was probably similar. The effect of NaCl on the physiology of the ponderosa pine was not instantaneous but occurred through time. The large difference between photosynthetic rates of control plants and plants receiving irrigations of low osmotic potential solutions was probably a result of plant water potential differences. Boyer and Bowen (1970) found nonstomatal effects of water stress on sunflower (Helianthus annuus). The water stress affected chloroplast function and as stress increased there was a subsequent decrease in CO_2 assimilation which appeared to parallel the loss in Hill activity.

The great difference of leaf resistances between PEG- and NaCl-irrigated ponderosa pine may be evidence of a direct ion effect. Xylem water potentials of plants from both of these irrigation treatments averaged -12 bars. Sodium chloride possibly caused the partial closure of stomates. However, the exact effect of the sodium or chloride ion is not known. The chloride ion may have interferred with potassium balance in the guard cells, resulting in closure of stomata. It is also possible that as cells of ponderosa pine receiving NaCl irrigation accumulated ions, osmotic adjustment of guard cells by potassium was not enough to increase turgor pressure for opening of the guard cells.

SUMMARY AND CONCLUSIONS

Two experiments were conducted to determine the effect of sodium chloride (NaCl) on different soil and plant variables. Potted ponderosa pine (Pinus ponderosa) seedlings were treated with NaCl foliar sprays and irrigation treatments. The first experiment, Salt Toxicity Test 1, involved winter lath house treatments of 0, 3000, 6000, and 9000 ppm sodium chloride irrigations and foliar spray treatments. The second experiment, Salt Toxicity Test 2, was a greenhouse, sand-culture experiment in which treatment effects of equal osmotic potential solutions of polyethylene glycol (PEG) and NaCl were compared with each other and with control seedlings receiving a Hoagland's nutrient solution. Soil variables measured in both experiments were electrical conductivity of a soil extract (EC_e), soil pH, and levels of soil sodium and chloride. Plant variables measured in the first experiment were net photosynthetic and dark respiration rates, foliar injury, and concentration of sodium and chloride in the foliage. In Salt Toxicity Test 2, xylem water potential and leaf resistance were measured in addition to the same plant variables that were measured in Salt Toxicity Test 1.

Carbon dioxide exchange in Salt Toxicity Test 1 was measured using a closed system and an infrared gas analyzer. Contribution of CO_2 to the system from the soil was excluded by sealing the soil at the soil-atmosphere interface with a mixture of heavy mineral oil and parafin. In Salt Toxicity Test 2, the CO_2 exchange rates were measured using an open system of gas exchange and an infrared gas analyzer.

All sodium chloride irrigations of Salt Toxicity Test 1 caused severe foliar injury and almost total death loss during the experiment. Therefore, no CO_2 exchange data could be collected for these seedlings. The main effects of the combination of foliar spray and NaCl irrigation treatments significantly ($p < 0.01$) affected all soil and plant variables measured, except soil pH. Most of these treatment effects were caused by the sodium chloride irrigations.

Plants in Salt Toxicity Test 1 that received NaCl foliar sprays and distilled water irrigation treatments did not have significantly ($p > 0.05$) different net photosynthetic or dark respiration rates. However, an analysis of covariance showed that both soil chloride and soil sodium caused a significant ($p < 0.05$) decrease in net photosynthetic rates. The decrease in net photosynthetic rates caused by soil chloride and soil sodium may be evidence of direct ion toxicity. Soil concentrations of both these ions were probably too low to cause a decrease in soil solute potential or cause a nutritional problem by competing with other ions for plant uptake. The decrease in photosynthetic rates was not combined with a similar increase in foliar injury. This injury, therefore, may be occurring near highways that receive salts during the winter, thereby lowering plant vigor and increasing the chance of injury from other causes.

In Salt Toxicity Test 2, the CO_2 exchange rates were not significantly ($p > 0.05$) affected by either NaCl foliar sprays or solutions of differing osmotic potentials when applied as irrigation treatments. Electrical conductivity of the soil extract, levels of soil and foliar sodium, levels of soil and foliar chloride, and leaf resistance were

significantly ($p < 0.05$) increased by NaCl irrigations. Both sodium chloride and PEG irrigations significantly ($p < 0.05$) decreased xylem water potential, but were not different from each other. The large differences in leaf resistance among plants from the PEG and NaCl irrigation treatments was considered to be possibly an ion effect or intercellular osmotic effect. Potassium is important in increasing turgor pressure of the guard cells and chloride ions have been found to balance the level of potassium in guard cells. Therefore, concentrations of chloride in cells near the guard cells may interfere with potassium movement into the guard cells.

The effects of NaCl on ponderosa pine from both salt toxicity tests suggests that there is evidence to support the theory of direct ion toxicity. There is also evidence to support the idea that a decrease in net photosynthesis without a subsequent increase in foliar injury could occur.

Problems and Future Needs for Research

Most problems encountered during this project were associated with the CO_2 exchange measurements. Construction of a gas exchange system was costly and the gas analyzer required very expensive repairs. Temperature control at irradiances above 0.30 ly $\cdot$ min^{-1} was difficult for the open system. Higher light intensity would be preferred for future CO_2 analysis.

Research should be continued to locate the effect of NaCl on leaf resistance. The effect of small concentrations of NaCl (< 8000 ppm) on leaf resistance, over long periods of time, should also be investigated. Possible effects of NaCl on leaf resistance to be investigated should be membrane changes and levels of plant hormones, especially kinetin and abscissic acid.

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APPENDIX TABLES

Table 1. Analyses of variance for the effects of sodium chloride foliar spray and irrigation treatments on some foliage characteristics of ponderosa pine (Pinus ponderosa).

Source of variation	d.f.	Mean squares	
		Foliar chloride	Foliar sodium
Treatment	6	1651671460***	452186609***
Foliar spray (F)	3	63014787***	10212192*
Irrigation (I)	3	3238650607***	887995400***
F x I	9	66024384***	14295821**
Error	63	8924238	4552627
Total	78	141877887	41045951

Source of variation	d.f.	Mean squares			
		Injury estimate Feb. 12, 1976	Injury estimate April 1, 1976	Injury estimate May 31, 1976	Injury estimate June 25, 1976
Treatment	6	15.8***	11265***	16050***	17831***
Foliar spray (F)	3	14.9**	1055*	256	132
Irrigation (I)	3	16.9**	21184***	31709***	35429***
F x I	9	6.1*	925*	359	77
Error	63	3.3	439	247	225
Total	78	4.6	1352	1507	1598

*** indicates significant difference at the 0.01 level of probability.
** indicates significant difference at the 0.05 level of probability.
* indicates significant difference at the 0.10 level of probability.

Table 2. Analyses of variance for the effects of sodium chloride foliar spray and irrigation treatments on some soil characteristics of ponderosa pine (Pinus ponderosa).

Source of variation	d.f.	Mean squares			
		Soil chloride	Soil sodium	pH	Electrical conductivity
Treatments	6	13602049***	12024761***	0.161	348.3***
Foliar spray (F)	3	101789	25505	0.290**	2.9
Irrigation (I)	3	27107410***	24018926***	0.032	693.6***
F x I	9	296844**	240523**	0.195**	7.8**
Error	63	148468	103212	0.084	3.7
Total	78	1200479	1036097	0.103	30.7

*** indicates significant difference at the 0.01 level of probability.
** indicates significant difference at the 0.05 level of probability.

Table 3. Analyses of variance for the effects of sodium chloride foliar spray on net photosynthesis and aboveground dark respiration of ponderosa pine (Pinus ponderosa).

Source of variation	d.f.	Mean squares			
		Photosynthesis (green)	Photosynthesis (total)	Respiration (green)	Respiration (total)
Treatment	4	0.4406	0.5893*	0.0906	0.0766
Error	19	0.2906	0.2596	0.0917	0.0613
Total	23				

* indicates significant difference at the 0.10 level of probability

Table 4. Analyses of covariance for the effect of soil sodium and foliar spray on net photosynthesis and dark respiration (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$) of ponderosa pine (Pinus ponderosa).

Source of variation	d.f.	Mean square			
		Photosynthesis (green)	Photosynthesis (total)	Respiration (green)	Respiration (total)
Covariate (soil sodium)	1	1.460**	2.189**	0.010	0.002
Main effect (NaCl foliar spray)	4	0.185	0.248	0.092	0.076
Error	18	0.283	0.240	0.096	0.065
Total	23				

** indicates significant difference at the 0.05 level of probability.

Table 5. Analyses of covariance for the effect of soil chloride and foliar spray on net photosynthesis and dark respiration (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$) of ponderosa pine (Pinus ponderosa).

Source of variation	d.f.	Mean square			
		Photosynthesis (green)	Photosynthesis (total)	Respiration (green)	Respiration (total)
Covariate (soil chloride)	1	1.375**	2.299**	0.004	0.000
Main effect (NaCl foliar spray)	4	0.155	0.159	0.091	0.077
Error	18	0.294	0.254	0.097	0.065
Total	23				

** indicates significant difference at the 0.05 level of probability.

Table 6. Analyses of variance for the effects of sodium chloride foliar spray on some soil characteristics for ponderosa pine (Pinus ponderosa).

Source of variation	d.f.	Soil chloride	Soil sodium	pH
Treatment	4	3371**	324**	0.2419**
Error	20	1070	108	0.0478
Total	24			

Source of variation	d.f.	Electrical conductivity
Treatment	4	0.0164
Error	20	0.0658
Total	24	

** indicates significant difference at the 0.05 level of probability.

Table 7. Analyses of variance for the effect of different osmotic potential solutions on net photosynthesis and dark respiration (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$) of ponderosa pine (Pinus ponderosa)

Source of variation	d.f.	Mean square	
		Photosynthesis	Respiration
Replications	5	59.101***	0.919**
Osmotic solutions (O)	4	13.383	0.101
Error I	24	14.919	0.301
Days (D)	3	4.422***	0.525***
O x D	12	1.829*	0.070
Error II	71	0.388	0.047
Total	95		

*** indicates significant difference at the 0.01 level of probability.
** indicates significant difference at the 0.05 level of probability.
* indicates significant difference at the 0.10 level of probability.

Table 8. Analyses of variance for the effect of different osmotic potential solutions on some soil characteristics for ponderosa pine (Pinus ponderosa).

Source of variation	d.f.	Soil chloride	Soil sodium	pH
Treatment	4	26489759**	18793192***	0.03
Error	15	673407	412665	0.04
Total	19			

Source of variation	d.f.	Electrical conductivity
Treatment	4	428***
Error	15	8
Total	19	

*** indicates significant difference at the 0.01 level of probability.

Table 9. Analyses of variance for the effect of different osmotic potential solutions on some plant characteristics of ponderosa pine (Pinus ponderosa).

Source of variation	d.f.	Foliar chloride	Foliar sodium
Treatment	4	9171121***	967804***
Error	25	562648	37244
Total	29		

Source of variation	d.f.	XWP[1] constant light	XWP 24 h dark	Leaf resistance	Leaf conductance
Treatment	4	19.9***	26.8***	219**	0.0015*
Error	10	1.6	1.3	13	0.0005
Total	14				

[1]XWP = xylem water potential
*** indicates significant difference at the 0.01 level of probability.
** indicates significant difference at the 0.05 level of probability.
* indicates significant difference at the 0.10 level of probability.